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NEURAL AND ENDOCRINE MECHANISMS INVOLVED IN PHYSIOLOGICAL AND BEHAVIORAL RESPONSES OF THE REPRODUCTIVE SYSTEM TO PHOTOPERIOD IN FEMALE HAMSTERS (Mesocricetus auratus)

By

Lori Linn Badura

A DISSERTATION

Submitted to
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Department of Psychology and Neuroscience Program

1988

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ABSTRACT

NEURAL AND ENDOCRINE MECHANISMS INVOLVED IN PHYSIOLOGICAL AND BEHAVIORAL RESPONSES OF THE REPRODUCTIVE SYSTEM TO PHOTOPERIOD IN FEMALE HAMSTERS (Mesocricetus auratus)

By

Lori Linn Badura

Reproductive responses of hamsters to photoperiod are mediated by a multisynaptic neural pathway that conveys photic information from the retina to the pineal gland. Many of the functional and anatomical descriptions of this pathway have been based upon work conducted with the male hamster. The present series of experiments sought to expand our current knowledge of this system by investigating the functional neuroanatomy that mediates behavioral, physiological, and neuroendocrine responses to photoperiod in females.

Similar to previous reports for males, horizontal knife cuts placed between the suprachiasmatic (SCN) and paraventricular (PVN) nuclei of the hypothalamus prevented the effects of inhibitory photoperiods on gonadal physiology. Thus, the connections between the SCN and the PVN appear to be important for mediating reproductive responses to photoperiod. However, these connections do not appear to be necessary for the expression of all photoperiod-dependent reproductive responses. These knife cuts did not prevent the short-day induced decrease in behavioral sensitivity to exogenous ovarian steroids.

Pinealectomy abolishes gonadal responses to short days, but does not prevent the decrease in behavioral sensitivity to estradiol seen when females are maintained in a nonstimulatory photoperiod. Likewise, administration of the pineal product, melatonin, alters gonadal, but not behavioral, responses to photoperiod. These findings suggest that photoperiodic effects on hormonal activation of sexual behavior are mediated by a neural system distinct from that involved in other reproductive responses.

Previous reports have indicated that horizontal knife cuts placed just dorsal to the PVN prevent short-day induced testicular regression in males. However, similar knife cuts in the present investigation did not abolish gonadal responses to photoperiod in females. Differential responsiveness to the disruption of a neural mechanism that modulates the release of follicle-stimulating hormone (FSH) may account for this sex difference.

Knife cuts that interrupt connections between the SCN and PVN disrupt the temporal characteristics of FSH secretion. The FSH peak in animals with knife cuts in short days is advanced with respect to that shown by neurally intact animals in the same photoperiod. The earlier FSH peak may reflect a disruption of the transfer of photic and circadian information to the pineal gland.

For my family,
who have always been there,
and have always believed.

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INTRODUCTION

In many mammalian species, seasonal reproductive cycles are generated by an interaction between photoperiod and the organism's endogenous circadian system (see Elliot & Goldman, 1981, for a review). Responses of the reproductive system to photoperiod are dependent upon the integration of photic and circadian information by a neural mechanism of which the suprachiasmatic nuclei (SCN) of the hypothalamus are an integral component. The SCN receive photic information via direct retinal input, and relay this information to the pineal gland through a multisynaptic pathway that includes connections with other hypothalamic sites. The neural input is ultimately converted by the pineal gland into an endocrine signal, melatonin, which induces the appropriate response of the pituitary-gonadal axis to photoperiod.

In male golden hamsters (Mesocricetus auratus), exposure to less than 12.5 hrs of light per 24-hr day results in testicular regression and cessation of spermatogenesis (Gaston & Menaker, 1967). Changes in circulating levels of gonadotropins precede testicular regression (Tamarkin, et al., 1976b), with a decrease in serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) occurring within 10 days of transfer from long to short days. Exposure to short days also induces an increase in sensitivity of the hypothalamic-hypophysial-gonadal axis to the negative feedback effects of steroid hormones. Low concentrations of testosterone (T) are more effective in suppressing post-castration levels of LH and FSH in animals exposed to short days (Tamarkin, et al., 1976a). An increase in the sensitivity of the hypothalamic-hypophysial axis to the negative feedback effects of T in males is also seen in animals treated with the pineal product, melatonin (Sisk & Turek, 1982). The physiological effects of exposure to short days are accompanied by a decline in copulatory behavior as a result of decreasing levels of T, although a nonstimulatory photoperiod also appears to reduce the behavioral responsiveness of the animals to T. Copulatory behavior can be restored in castrated males by T replacement, however,

larger doses of T are required by animals in short days (Campbell, et al., 1978; Morin & Zucker, 1978).

The mechanisms by which nonstimulatory photoperiods affect the reproductive system of the female hamster have been less well documented. Females are rendered anovulatory by exposure to short days, and this process is accompanied by changes in the release of gonadotropins (Seegal & Goldman, 1975). Gonadally-intact females display a proestrus surge of LH and FSH once every 4 days (Albers, et al., 1985; Bast & Greenwald, 1974), while acyclic animals display a daily afternoon rise in both of these hormones (Albers, et al., 1985). Females also show a decline in sexual receptivity that is related to regression of the gonads with prolonged exposure to short days. Unlike the male, however, photoperiod differences in the ability of steriod hormones to facilitate sexual behavior have not been reported.

Estrous cyclicity in both the hamster and the rat is a noncircadian event that nonetheless depends upon the circadian system (Fitzgerald & Zucker, 1976; Swann & Turek, 1985). The SCN are involved in the generation of circadian rhythms and interact with multiple neural and neuroendocrine systems to modulate ovulation both in photoperiodic and nonphotoperiodic species. Lesions of the SCN abolish ovulatory cycles, as well as circadian rhythms in locomotor activity, feeding, drinking, and pineal N-acetyltransferase activity (Raisman & Brown-Grant, 1977; Stephan & Zucker, 1972; Stetson & Watson-Whitmyre, 1976). The estrous cycle of female rats (Nunez & Casati, 1979) and hamsters (Norman, et al., 1972) is suspended by retrochiasmatic knife cuts, but these cuts do not affect behavioral circadian rhythms (Nunez & Casati, 1979). Present knowledge concerning the role of many of these hypothalamic connections in the photoperiodic control of reproduction is incomplete however. One group of SCN efferent fibers that has received much attention is that which projects dorsally to the medial parvocellular region of the paraventricular nucleus (PVN) of the hypothalamus (Stephan, et al., 1981). In male rats, knife cuts that interrupt these

dorsal efferent projections but leave the SCN undamaged do not disrupt behavioral circadian rhythms (Brown & Nunez, 1986). Similar dorsal cuts in male hamsters abolish reproductive responses to photoperiod (Eskes & Rusak, 1985; Inouye & Turek, 1986; Nunez, et al., 1985) even though the entrainment of behavioral rhythms to light-dark cycles remains undisturbed (Nunez, et al., 1985).

A similar reproductive insensitivity to changes in photoperiod is observed after lesions of the SCN (Rusak & Morin, 1976) or the PVN (Pickard & Turek, 1983). The PVN send long projections to the spinal cord (Don Carlos & Finkelstein, 1987). These projections may modulate sympathetic outflow to the pineal gland. Two distinct bundles of PVN efferent projections to the spinal cord have been described in the rat (Luiten, et al., 1985): one that takes a dorsal-caudal path (Tract I) and one that exits the PVN in a lateral direction (Tract II). In hamsters, however, the path of these efferent projections has not been fully described.

The neural circuitry involved in photoperiodic modulation of reproduction in female hamsters is also unclear. Although studies to date have operated under the assumption that the system is the same in males and females, several findings suggest there may in fact be sex differences in the neural control of seasonal reproduction. Lesions of the SCN in males block the testicular regression induced by short days (Eskes, et al., 1984; Rusak & Morin, 1976; Stetson & Watson-Whitmyre, 1976). In contrast, lesions of the SCN in female hamsters result in an anovulatory state characterized by persistent vaginal and behavioral estrus (Stetson & Watson-Whitmyre, 1976). Since the acyclicity that follows lesions of the SCN occurs in females housed in both long and short days, it is difficult to evaluate effects of the lesions on sensitivity to photoperiod.

An apparent sex difference also exists in the ability of steroid hormones to restore sexual behavior in nonstimulatory photoperiods. As stated earlier, castrated male dorsal efferent projections but leave the SCN undamaged do not disrupt behavioral circadian rhythms (Brown & Nunez, 1986). Similar dorsal cuts in male hamsters abolish reproductive responses to photoperiod (Eskes & [illegible] hamsters housed in short days require much higher levels of T to reinstate copulatory behavior than

castrates kept in long days (Campbell, et al., 1978; Morin & Zucker, 1978). A photoperiod-dependent difference has not been reported for the ovariectomized female hamster with respect to estrogen (E)/progesterone (P) facilitation of sexual receptivity (Morin, 1982; Zucker, et al., 1979). However, this failure to find photoperoidic effects on sensitivity to ovarian hormones might be a function of the steroid replacement paradigm employed to induce sexual receptivity. Sexual receptivity has been induced in females housed in long days with estradiol benzoate (EB; Carter, et al., 1973) or free estradiol (Meisel & Sterner, 1986) alone. A differential sensitivity to E across photoperiods may exist, and may play an important role in the control of seasonal reproduction in females by decreasing the likelihood of displays of sexual behavior during inappropriate seasonal conditions.

Interpretation of anatomical studies concerning the neural mechanisms involved in the control of seasonal reproduction has been based upon the assumptions that seasonal and nonseasonal species, as well as males and females, rely upon the same pathways for transduction of photic information. The comparative functional anatomy of this system deserves further attention before conclusions can be drawn across species or gender. The goal of the dissertation work described here was to further investigate the functional anatomy of the neural system involved in the modulation of reproductive responses to photoperiod in the female hamster.

EXPERIMENT 1

Knife Cuts Ventral to the PVN and Reproductive Responses to Photoperiod

Lesions of the PVN and the SCN abolish photoperiod-dependent gonadal responses to photoperiod in both male and female hamsters (Brown, et al., 1988; Bartness, et al, 1985; Lehman, et ., 1984; Pickard & Turek, 1983; Stetson & Watson-Whitmyre, 1976). In contrast to SCN lesions, however, PVN damage does not abolish estrous cyclicity (Bartness, et al., 1985) or circadian rhythms of locomotor activity (Pickard & Turek, 1983). In male hamsters, horizontal knife cuts that interrupt the connections between the SCN and the PVN also prevent the gonadal regression that is induced by exposure to short days (Eskes & Rusak, 1985; Inouye & Turek, 1986; Nunez, et al., 1985). In the present experiment, female hamsters were given similar knife cuts and were monitored for potential photoperiod-induced changes in estrous cyclicity. In addition, the animals were ovariectomized and tested for potential photoperiod-dependent changes in sensitivity to exogenous ovarian hormones.

Methods

Subjects and Housing

Adult female hamsters (LVG/LAK; Charles River Breeding Laboratory, Newfield, NJ) weighing 90-100 g were individually housed in plastic cages (24.0 x 18.0 x 13.0 cm) and maintained with food (Wayne: Mouse Breeder Blox) and water available ad lib under a long-day (16L:8D; lights out at 1800 hr) photoperiod (illumination approximately 15 ftc.). Adult male hamsters for use as stimuli in the behavioral testing were group housed in plastic cages (34.0 x 29.5 x 16.5 cm; n=6/cage) in the same colony room. For all females, vaginal discharges were evaluated daily and used to monitor estrous cyclicity (Orsini, 1961). Body weight (nearest g) was recorded weekly. Only females that showed at least three consecutive 4-day estrous cycles prior to surgery were used in the experiment.

Surgical Procedure

One group of hamsters (n=15) was anesthetized with Equithesin (4.5 ml/kg) between 0700 and 1300 hr on the day of proestrus and placed in a stereotaxic apparatus (Kopf Instruments) with the incisor bar 2.0 mm below ear bar zero. A flap of skull (3 mm square) was removed, the superior sagittal sinus retracted, and a Scouten microknife (Scouten, et al., 1981) lowered through the brain 6.6 mm ventral to the sinus and 0.3 mm posterior to the bregma to a point aimed midway between the PVN and the SCN. The wire was then extended 2.0 mm from the barrel and rotated 360o in both directions. Another group of females (n=17) received a sham surgical procedure identical to that of the knife cut group except that the blade was not extended.

All animals were returned to the 16L:8D photoperiod for 1 week and allowed to recover from surgery. On the morning of proestrus the following week, eight of the animals with knife cuts and nine of the sham-operated animals were moved to a nonstimulatory (6L:18D) photoperiod with lights off at 1800 h. All animals remained in the assigned photoperiods for the duration of the experiment.

Estrous Cyclicity and Ovariectomy

The vaginal discharge of each female was inspected daily until all sham-lesioned animals in 6L:18D showed discharges characteristic of diestrus for at least two weeks. Cycling females were then bilaterally ovariectomized (OVX) under Equesthesin anesthesia on the morning of proestrus; sham-operated anestrous animals were OVX at random times over the four days. Before OVX, the uteri were exposed, positioned on a white card next to a metric ruler, and photographed using a Polaroid Land camera mounted on a copy stand. Uterine width measurements to the nearest 0.1 mm for each animal were taken from the Polaroid photographs from one uterine horn by two raters with no information about the animal's reproductive status at the time of OVX. A correlation of the two raters' scores revealed a high degree of agreement (Pearson's r = 0.96). An average score was used in those cases where measurements differed. Following OVX, the animals were returned to the respective photoperiods and permitted to recover for 3 weeks.

Sexual Behavior Testing

Following the 3-week recovery period, all females received daily subcutaneous injections of 6 μg of estradiol benzoate (EB; Sigma Chemical Co.) in 0.1 ml of sesame oil for 12 days. A similar dose of EB has been shown to induce lordosis in female and male hamsters housed under long-day conditions (Carter, et al., 1973). Behavioral testing was conducted under dim red illumination in 5-min individual sessions after 9 and 11 days of treatment with EB using adult male hamsters (140-160 g) as stimuli. Testing began at 1800 hr for animals in 16L:8D and at 100 hr for animals in 6L:18D.

These times have been shown to correspond with the onset of activity for each photoperiod as determined by running wheel records (see Elliot & Goldman, 1981, for a review), and results from our laboratory indicate that these are optimal times for behavioral testing under these two photoperiods (Badura, unpublished findings). The procedure was then repeated on day 12 of EB treatment with all females receiving a subcutaneous injection of 0.25 mg of progesterone (P; Sigma Chemical Co.) in 0.1 ml of sesame oil 4 hr prior to testing. On all testing days, latency to show lordosis was recorded with a hand-held timer. Each test began when the stimulus male was placed in the female's cage, and was terminated after the onset of lordosis, or after 5 min for those animals that did not show lordosis behavior. All animals in 6L:18D had been exposed to short days for 17 weeks at the conclusion of behavioral testing.

Histology

Two animals in 6L:18D and one animal in 16L:8D died before the beginning of behavioral observations. The brains from these animals were removed and immersion-fixed in a 30% sucrose-10% formalin solution and stored at 4 ^{o} C for 2 weeks prior to frozen sectioning. After behavioral testing was completed, all remaining animals with knife cuts were sacrificed via anesthetic overdose and perfused transcardially with 0.9% saline followed by 10% formalin. The brains were removed and stored in a 30% sucrose-10% formalin solution at 4 ^{o} C for at least 1 week. Frozen sections at 40 µm were mounted on slides and counterstained with cresylecht violet for microscopic evaluation.

Statistics

Initial between group comparisions for both the percentage of animals showing estrous cycles at the end of the 10 weeks, and the percentage of animals showing lordosis under each hormone treatment, were made using $\underline{X}^2$ analyses. Follow-up tests of selected comparisons were made using Fisher's exact probability tests. Between group comparisons for body weight and uterine width data were made using

These times have been shown to correspond with the onset of activity for each photoperiod as determined by running wheel records (see Elliot & Goldman, a review).

two-way analyses of variance (ANOVA; photoperiod x surgical condition), followed by a series of modified t-tests (Winer, 1962, p.208).

Results

Placement of Knife Cuts

Placement of the knife cuts was determined by an investigator unaware of the group assignment or reproductive status of each animal. The glial scar resulting from a representative knife cut is shown in Figure 1. In all except seven cases, knife cuts in animals in both 6L:18D (n=8) and 16L:8D (n=6) photoperiods were bilateral and confined to an area dorsal to the SCN and ventral to or through the PVN. One animal in 6L:18D had a unilateral knife cut with a contralateral mechanical lesion of the PVN. This animal continued cycling and was included in all of the analyses. One animal from each photoperiod group sustained unilateral damage through or ventral to the PVN. Four animals from 6L:18D possessed knife cuts located just dorsal to the PVN, and these cuts did not prevent short-day-induced acyclicity or uterine regression. These six animals were not included in the analyses. Figure 2 depicts schematically the locations of the cuts for the remaining animals in 16L:8D and 6L:18D.

Cyclicity

All groups of females continued to show 4-day estrous cycles during the 1-week postoperative recovery period in 16L:8D. The animals with bilateral knife cuts ventral to or through the PVN that were transferred to 6L:18D continued to show regular estrous cycles for the next 10 weeks. Animals with similar knife cuts and sham-operated animals that remained in 16L:8D also continued to cycle, except for one female with a knife cut (No. 12) that ceased cycling after 4 weeks and showed discharges characteristic of diestrus throughout the rest of the experiment. In contrast, the majority of sham-operated control animals in 6L:18D became acyclic after 8 weeks and none showed estrous cycles after 10 weeks. An analysis of the percentage of cycling animals at the end of the 10 weeks revealed an overall significant difference

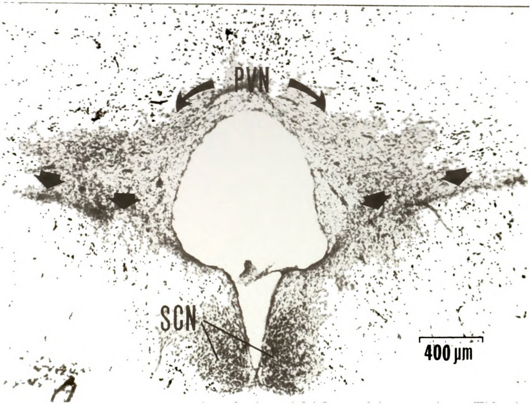

Figure 1. Photomicrograph of a coronal section (40µm thick, cresylecht violet stain) through the hypothalamus of a hamster (No. 1) in which the knife cut (indicated by arrows) produced bilateral damage just ventral to the PVN.

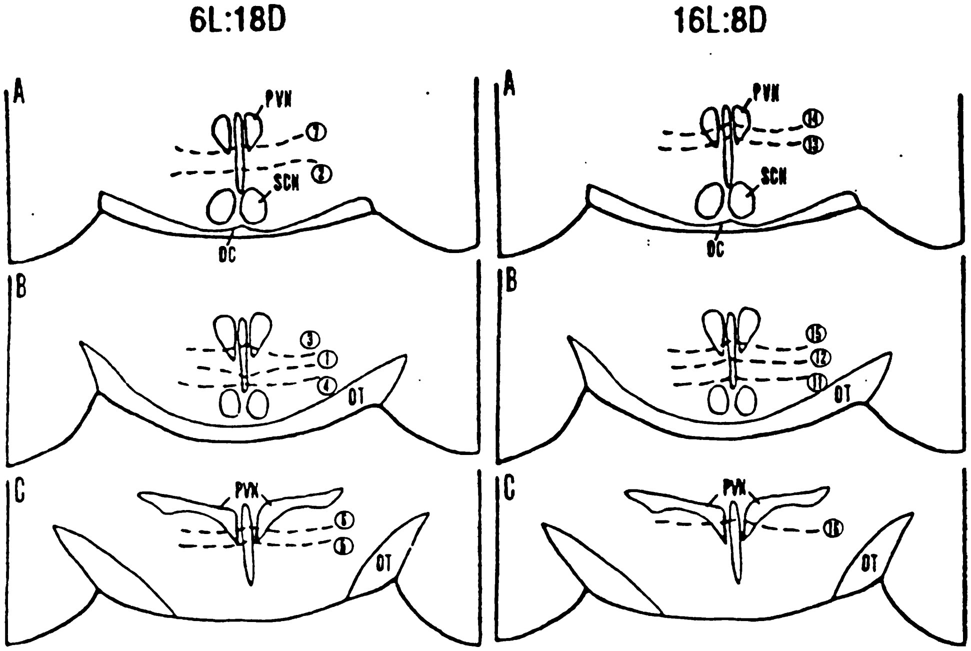
6L:18D
16L:8D
A
PVN
⑦
②
SCN
OC
B
③
①
④
OT
C
PVN
⑤
⑥
OT
A
PVN
⑭
⑬
SCN
OC
B
⑮
⑫
⑪
OT
C
PVN
⑯
OT

Figure 2. Schematic representation of knife cuts that produced bilateral damage to the hypothalamus for animals in 16L:8D and 6L:18D photoperiods. The cuts (dashed lines) are shown at the point of greatest bilateral damage for each case using drawings (a-c) modified from those published by Lehman, et al. (1984). The individual cases are identified by numbers (No. 1,7, and 15 died before sexual behavior testing). One animal from the 6L:18D group (not shown) had a unilateral knife cut combined with a contralateral mechanical lesion of the PVN. OC=optic chiasm, OT=optic tract.

across groups ($\underline{X}2 = 27.22$, $\underline{p} < 0.001$; Figure 3). Follow-up analyses indicated that the percentage of animals cycling in the knife cut groups in both the 16L:8D and 6L:18D photoperiods, and of sham-operated animals in 16L:8D differed from the sham-operated animals in 6L:18D ($\underline{p} = 0.002$, $\underline{p}=0.004$, and $\underline{p}=0.004$, respectively).

Body Weight and Uterine Width

Body weights did not differ significantly across photoperiod group or surgical condition before OVX, nor was body weight significantly affected following EB treatment for 11 days (Table 1). A comparision of uterine width measurements across groups revealed a significant interaction of photoperiod and surgical condition ($\underline{F}$ (1,23) = 5.95, $\underline{p} < 0.05$; Table 1). There were also significant main effects for both photoperiod ($\underline{F}$ (1,23) = 19.68, $\underline{p} < 0.001$) and surgical condition ($\underline{F}$ (1,23)= 39.06, $\underline{p} < 0.001$). Follow-up analyses found significant differences in uterine width between sham-operated animals in 6L:18D and all other groups as follows: animals with knife cuts in 6L:18D ($\underline{t}$ (23) = 6.26, $\underline{p}$ 0.001), animals with knife cuts in 16L:8D ($\underline{t}$ (23) = 7.38, $\underline{p} < 0.001$), and sham-operated animals in 16L:8D ($\underline{t}$ (23) = 5.43, $\underline{p} < 0.001$). Thus, acyclic animals in 6L:18D had significantly smaller uterine widths than any of the other groups.

across groups (X2 = 27.22, p < 0.001; Figure 3). Follow-up analyses indicated that the percentage of animals cycling in the knife cut groups [illegible]

6L:18D [illegible]

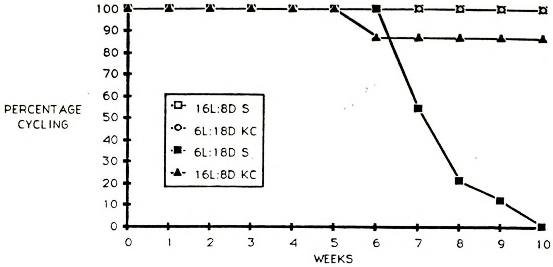

Figure 3. Percentage of animals with knife cuts (KC) or sham surgery (S) showing normal estrous cycles in each photoperiod for each week (16L:8D KC, n=6; 16L:8D S, n=8; 6L:18D KC, n=7; 6L:18D S, n=9). The 6L:18D S group was significantly different from all other groups ($p < 0.0002$ for all comparisons).

Table 1

Mean (± SEM) body weight before (pre-EB) and after 11 days of EB treatment (post-EB) and uterine width at the time of ovariectomy for each treatment group in Experiment 1.

		Body Weight (g)		Uterine
Group	n	pre-EB	post-Eb	width (mm)
16L:8D KC	5	162.2 ± 5.2	157.6 ± 5.4	3.8 ± 0.08
16L:8D S	7	157.3 ± 2.9	157.9 ± 2.8	3.2 ± 0.04
6L:18D KC	5	149.4 ± 4.2	140.8 ± 3.9	3.5 ± 0.11
6L:18D S	7	156.7 ± 2.0	155.7 ± 2.2	2.1 ± 0.05 a

KC = knife cuts; S = sham surgery

a Significantly different from all other groups (p < 0.001 for all comparisons

Sexual Behavior

Within all groups, the percentage of animals showing lordosis after 9 or 11 days of EB treatment was not significantly different ($X2$, $p > 0.05$); thus, data from these two testing days were collapsed for the analyses.

Collapsing across surgical condition, there was a significant overall effect of photoperiod on the percentage of animals responding to EB treatment alone ($X2 = 5.06$, $p < 0.05$; see Table 2). A greater percentage of animals in 16L:8D responded to EB stimulation. When P was administered, however, the effect of photoperiod was not present ($X2 = 0.89$, $p > 0.05$). The difference in sensitivity to EB in the facilitation of sexual behavior was independent of surgical condition. None of the animals with knife cuts that had been cycling in 6L:18D (n=5) responded to the EB treatment alone. For those animals responding to hormone treatment, latency to show lordosis was not significantly different across groups.

Table 2

Percentage of animals in each photoperiod showing lordosis in response to EB and to EB + P treatment in Experiment 1

		Hormone Treatment	
Group	n	9 and 11 days EB [a]	12 days EB + P
16L:8D KC	5	40	100
16L:8D S	7	57	100
6L:18D KC	5	0	100
6L:18D S	7	14	86

[a] Significant effect of photoperiod for percentage of animals responding to EB alone ($p < 0.001$). The percentages are based on the number of animals that responded in at least one of the two tests.

Discussion

The present findings support the current model describing hypothalamic pathways involved in the photoperiodic regulation of reproductive responses (Tamarkin, et al., 1985). As in males, horizontal knife cuts placed dorsal to the SCN and ventral to or through the PVN abolish photoperiod-dependent gonadal responses in female hamsters. Animals with these knife cuts continue to show regular 4-day estrous cycles under short day conditions, suggesting that dorsal projections from the SCN to the area of the PVN are an important component of the neural mechanism modulating pineal responses to photoperiod, but are not necessary for the display of estrous cycles. The effectiveness of knife cuts between the SCN and the PVN in blocking gonadal responses to photoperiod is most likely due to a disruption of SCN projections to PVN neurons, and not SCN projections traveling through the PVN to terminate in more dorsal sites. Chemical lesions that destroy PVN neurons, but presumably leave fibers of passage intact, also inhibit gonadal regression in male hamsters maintained under short day conditions (Brown, et al., 1988).

Knife cuts placed between the SCN and the PVN also prevented the effects of exposure to short days on uterine size. Light deprivation induces uterine regression in neurally intact female hamsters, and removal of the pineal gland abolishes this response (Reiter, 1968). The ability of knife cuts to prevent uterine regression under short-day conditions further supports the importance of SCN-PVN connections in the neural control of the pineal gland. In the rat, the SCN sends a massive projection to the sub-PVN area (Watts & Swanson, 1984). It is not known if such a projection exists in the hamster. The sub-PVN area does receive a direct projection from the retina (Youngstrom, et al., 1987; Pickard, 1982) which may be involved in the photoperiodic control of pineal function.

Castrated male hamsters exposed to short days are less sensitive to exogenous T for inducing copulatory behavior than castrated males exposed to long days (Campbell, et al., 1978; Morin & Zucker, 1978). A similar effect of photoperiod on sensitivity to E and P in the facilitation of female sexual behavior has not been previously reported (Morin, 1985; Zucker, et al., 1980). The observations of sexual behavior in this study indicate that OVX females in short days are less sensitive to the activational effects of EB alone in inducing sexual receptivity. This effect of photoperiod is present even though the animals may already be experiencing the onset of neuroendocrine changes that accompany gonadal recrudescence after prolonged exposure to short days. Differences across photoperiod are not present when P is administered in conjunction with EB. This finding suggests that past failures to observe the effects of photoperiod on the steroid facilitation of female sexual behavior (Morin, 1985; Zucker, et al., 1980) may be due to a masking effect of P.

The mechanism modulating the photoperiodic control of the activational effects of E is not known. However, results from experiments with male hamsters suggest that photoperiod-induced differences in behavioral sensitivity do not involve changes in the concentration of neural E receptors (Callard, et al., 1986). However, it should be noted that these neural E receptors were measured only in whole tissue blocks from limbic and hypothalamic regions. Since this technique only detects the sum total of receptors within the tissue block, photoperiod-induced increases or decreases may have occurred in discrete nuclei and been obscured in comparison with changes in the rest of the block.

In the present study, knife cuts that block the gonadal responses to photoperiod, presumably by disrupting a pathway that provides photic and/or circadian information to the pineal gland, did not affect photoperiod-induced changes in the activational effects of EB on sexual behavior. While cuts ventral to the PVN should have interrupted direct SCN input to the PVN, they probably left SCN connections with

other hypothalamic sites intact. These sites may in turn transfer photic information to the pineal gland thereby inducing changes in pineal function. The expression of estrous cyclicity and sexual behavior may be sensitive to different parameters of pineal function. In Siberian hamsters, short-day induced pelage changes and testicular regression appear to be sensitive to different critical photoperiods (Duncan, et al., 1985). However, the possibility also exists that the neural mechanism responsible for the photoperiodic modulation of E-sensitivity may be independent of the pineal gland.

EXPERIMENT 2a

Photoperiod-dependent Changes in Behavioral Sensitivity to Ovarian Hormones

In Experiment 1, several potential confounds may have contributed to the finding of differential sensitivity to EB in the facilitation of sexual behavior across photoperiod. For instance, all of the stimulus males were housed in a long-day colony room with the time of lights out coincident with the time of testing for the long-day females, but 6 hr before the time of testing for the short-day females. These times represent different portions of the active phase of the circadian locomotor rhythm in the males, as defined by the time of activity onset for this long-day photoperiod. Thus, the circadian time at which the males were used as stimuli for each group differed, and potential differences in the levels of activity in the males may have promoted differential responding in the females. Similarly, the females in both photoperiod groups received the EB injections at the same clock time, but different circadian time, and the single injected dose of EB used made it difficult to draw more general conclusions concerning the sensitivity of animals in long and short days. Recently, Meisel & Sterner (1986) have induced lordosis in OVX female hamsters using subcutaneous implants of free estradiol (E). Thus, an experiment using silastic implants was designed in an effort to gain control over some of the confounding factors discussed above. In addition, aggressive behavior was monitored in each testing session to evaluate the possible photoperiodic modulation of hormonal effects on this social behavior as well.

Methods

Animals and Housing

Adult male and female hamsters (LVG/LAK) weighing 90-100 g were obtained from Charles River Breeding Laboratory (Newfield, NJ). All females were individually housed in plastic cages (24.o x 18.0 x 13.0 cm) with food and water available ad lib. The females were kept under either long (16L:8D; n = 8) or short (6L:18D; n=11) photoperiods for three weeks to allow adaptation to the laboratory conditions. Light intensity was similar for each photoperiod (approximately 15 ft-c.). Stimulus males were group-housed in plastic cages (34.0 x 29.5 x 16.5 cm; n=6/cage). The males were kept in long days (16L:8D) in two different colony rooms with the time of lights out coincident with the time of testing for the two groups of females.

Procedure

Body weights were recorded for each female weekly throughout the experiment. After the adaptation period, the females were bilaterally OVX under Equithesin anesthesia (4.5 ml/kg) and permitted to recover for 4 weeks. One week before testing, these animals received a 10 mm silastic capsule (3.175 mm o.d.; 1.575 mm i.d.) containing a crystalline mixture of 25% E (Sigma Chemical Co.) diluted with cholesterol. The capsules, without previous incubation, were implanted subcutaneously under Metofane (methoxyflurane; Pittman-Moore Co.) anesthesia. This dose of E has been shown to induce lordosis in 50-60% of animals housed in long days (Meisel & Sterner, 1986). The females were then tested for sexual behavior in their home cages with randomly chosen sexually active stimulus males (140-160 g at the time of testing) under dim red illumination. Each test started when the male was placed in the female's cage. The latency to show lordosis or aggressive behavior was recorded to the nearest second for each female using a hand-held timer. An aggressive display was defined as a biting attack initiated by either the male or the female (see

Floody & Pfaff, 1977, for a review). Tests were terminated immediately after the onset of lordosis or aggression, or after 5 min for animals that did not display either behavior.

In previous 5-min tests (Badura, unpublished observations), biting attacks were never followed by the display of lordosis.

Females in long days were tested shortly after lights out, whereas animals in short days were tested approximately 6 hr after lights out. After the tests, the capsules were removed and approximately two weeks allowed for clearance of E from the animal's systems. The procedure was then repeated but this time the females received capsules containing a mixture of 50% E diluted with cholesterol. One week after implantation, behavioral testing was conducted as described above. The following day, all animals received a subcutaneous injection of 0.25 mg of P in 0.1 ml of sesame oil 4 hr prior to pairing with a stimulus male, and the testing procedure was repeated.

Statistics

Comparisons based on percentage of animals responding employed nonparametric statistics; the Fisher's exact probability test was used for between-group comparisons, and within-group comparisons were evaluated using the McNemar test. The data on latency to respond and body weights were analyzed using the Student's t-test. Differences were considered significant when $p < 0.05$ (two tails).

Results

Figure 4 shows the percentage of animals from each photoperiod that showed lordosis while receiving each of the three hormone treatments. In both photoperiods, all females that showed lordosis in response to the hormone treatments did so prior to mounting by the male. A higher percentage of females responded to the low (25%) dose of E in long days that in short days ($p = 0.05$). A similar trend was observed when the 50% E dose was employed, but this effect of photoperiod failed to reach statistical significance ($p = 0.10$). No effects of photoperiod on this measure were seen

Floody & Pfaff, 1977, for a review). Tests were terminated immediately after the onset of lordosis or aggression, or after 5 min for animals that behavior.

In previous

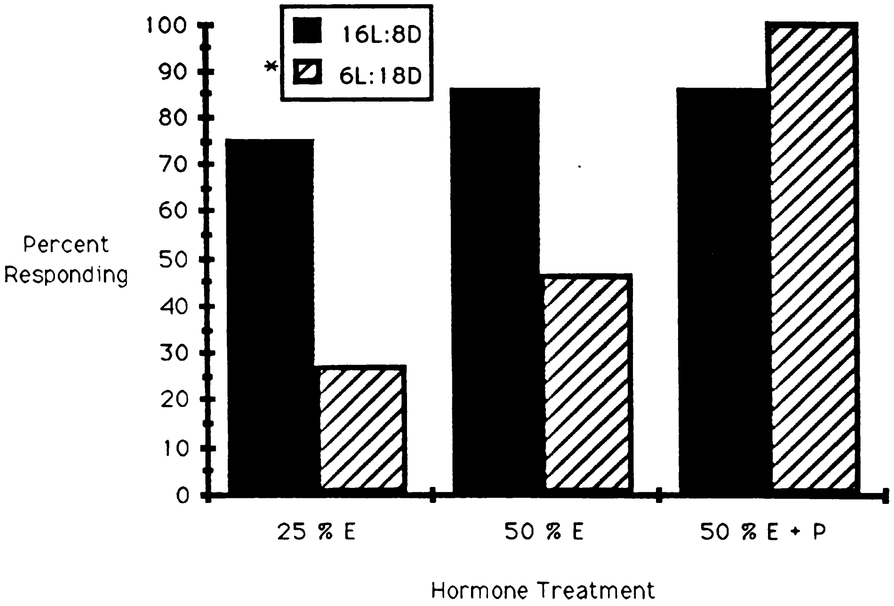

Figure 4. Percentage of animals in 16L:8D and 6L:18D responding with lordosis behavior to each dose of E and to E and P. *Significantly different from the 16L:8D group at the 25% E dose p = 0.05). **Significantly different from the two E doses within the 6L:18D group (p < 0.025 for both comparisons).

when the animals received E and P ($p = 0.43$). In long days, the percentage of animals showing lordosis did not differ significantly across hormone treatments ($p > 0.15$ for all comparisons). However, for the short-day group, both doses of E were significantly less effective in the facilitation of lordosis than the E and P treatment ($p < 0.025$ for both comparisons). For the animals responding, comparisons of latencies to show lordosis when treated with either dose of E or with E and P did not reveal significant differences across photoperiods or within groups ($p > 0.1$ for all comparisons; see Table 3).

All displays of aggression (i.e., biting attacks) were initiated by the female and occurred prior to attempts to mount by the male. While receiving the 25% E dose, the percentage of females showing aggressive behavior was significantly higher for the short-day group (73%) than for the long-day group (25%; $p = 0.05$; see Figure 5). This significant effect of photoperiod was not evident during the other two hormone treatments ($p > 0.16$ for both comparisions). In long days, the number of animals showing aggression was not differentially affected by hormone treatment ($p > 0.15$ for all comparisions); however, in short days, significantly more animals showed aggressive behavior when treated with either dose of E alone than when receiving E and P ($p < 0.05$ for both comparisons). The comparison of the two doses of E did not reach statistical significance ($p > 0.1$). Meaningful statistical comparisons of latencies to show lordosis or aggression were not possible due to the small number of animals showing these behaviors under many of the treatment conditions (see Table 3). Photoperiod and hormone treatments did not have significant effects on body weight ($p > 0.1$ for all comparisons; see Table 4).

Table 3

Mean (± SEM) latencies (sec) to show lordosis or aggression and number (n) of animals in each photoperiod responding for each hormone treatment in Experiment 2a

	Hormone Treatment		
Photoperiod	25% E	50% E	50% E + P
		Lordosis	
16L:8D	67.8 ± 29.03	6.2 ± 11.9	17.7 ± 4.5
n =	6	6	6
6L:18D	58.6 ± 40.3	26.2 ± 6.7	31.6 ± 7.4
n =	3	5	11
		Aggression	
16L:8D	162.5 ± 11.5	155.0 ± 0.0	42.0 ± 0.0
n =	2	1	1
6L:18D	145.8 ± 27.21	74.0 ± 35.4	----
n =	8	5	0

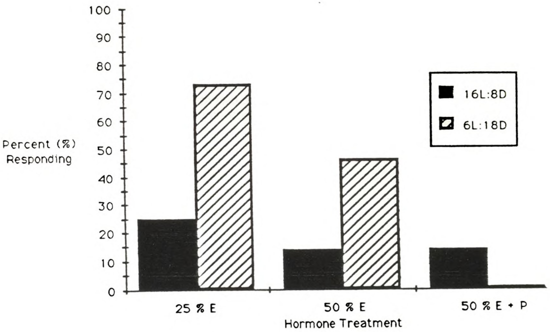

Figure 5. Percentage of animals in 16L:8D and 6L:18D responding with aggressive behavior to each dose of E and to E and P. *Significantly different from the 16L:8D group at the 25% E dose ($\underline{p} = 0.05$). **Significantly different from the two E doses within the 6L:18D group ($\underline{p} < 0.05$ for both comparisons).

Table 4.

Mean (± SEM) body weights (g) at the time of ovariectomy (initial), capsule implantation (pre-E), and after one week of treatment with two doses of estradiol (E) for animals in each photoperiod in Experiment 2a

Photoperiod	n	Initial	Pre-E	25% E	50% E
16L:8D	8[a]	105.1 ± 1.8	116.6 ± 4.5	117.1 ± 4.3	122.4 ± 5.3
6L:18D	11	101.1 ± 1.7	121.4 ± 3.9	125.5 ± 4.8	139.2 ± 8.2

[a] One animal died before the second capsule was implanted

Discussion

When exposed to a nonstimulatory photoperiod, ovariectomized hamsters showed a reduced sensitivity to the activational effects of E on lordosis behavior. The effects of photoperiod on body weight reported by others (Bartness & Wade, 1984) were not evident in this experiment, therefore, the differences in behavior reported here were not due to differences in the amount of hormone administered per gram of body weight. The behavioral effects of photoperiod seen in females parallels the short-day induced hyposensitivity to testosterone (T) previously observed in castrated male hamsters tested for copulatory behavior (Campbell, et al., 1978; Morin & Zucker, 1978). Aromatization of T to E has been implicated in the hormonal facilitation of male hamster sexual behavior (DeBold & Clemens, 1978; Lisk & Begier, 1980). Thus, a reduced behavioral sensitivity to E may also be present in male hamsters kept in short days. However, if this effect of photoperiod exists in the male, it is not associated with a significant reduction in neural E receptors (Callard, et al., 1986).

Previous work has shown that ovariectomized hamsters are less aggressive after E treatment when tested using males as stimuli (Ciaccio, et al., 1979; Payne & Swanson, 1971). Since our experimental procedure made aggression and lordosis incompatible responses, it is possible that photoperiod affected E-induced sexual receptivity by altering the suppressive effects of E on female aggressive behavior.

Interactions between photoperiod and E effects have also been seen in experiments on the circadian activity patterns of female hamsters (Widmaier & Campbell, 1980). Interestingly, the observed effects of long photoperiods on the behavioral actions of E are not always facilitative. Whereas E activation of lordosis (present data) and wheel running (Widmaier & Campbell, 1980) is enhanced by long photoperiods, some effects of E on the temporal distribution of activity are seen only when the animals are kept in

short days (Widmaier & Campbell, 1980). The mechanisms responsible for the photoperiodic modulation of steroid effects on behavior are not known; and may be independent of the pineal gland (see Experiment 1). Experiments with pinealectomized animals are needed to determine if behavioral and physiological effects of photoperiod share a common mechanism.

Past failures to detect photoperiodic effects on E and P facilitation of lordosis (Morin, 1985; Zucker, et al., 1980) may be due to the masking effects of the relatively large doses of P used. In the present experiment, a single large dose of P completely abolished the group differences seen when E alone was administered. It is possible that photoperiodic influences on behavioral sensitivity to E and P treatment would be revealed by the use of low doses of both hormones. Changes in behavioral sensitivity to steroids may contribute to the termination of sexual behavior induced by short photoperiod in both male and female hamsters.

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EXPERIMENT 2b

Behavioral Sensitivity to Ovarain Hormones: Role of the Pineal Gland

In Experiment 2a, the short- day induced decrease in sensitivity to the stimulatory effects of E on sexual behavior was not present after administration of P. However, the dose of P employed was probably supraphysiological. In an intact female exposed to short days, daily diurnal rises in P levels precede the onset of vaginal acyclicity. These surges of P are maintained throughout the period of anestrous together with low baseline levels of E (Bridges & Goldman, 1975; Jorgenson & Schwartz, 1985), yet these animals do not display sexual behavior. One way in which elevated levels of P in an intact anestrus female may be incapable of inducing sexual receptivity may be related to the photoperiod-induced decrease in sensitivity to E. Estrogen induces the production of progestin receptors in the brain (Clark, et al., 1982). Since sensitivity to E decreases in short days, there could be a reduction in the number of E-induced progestin receptors, and hence, a reduction in the sensitivity to P.

Another potential explanation for the inability of endogenous P in anestrus hamsters to facilitate receptive behavior may be the possible down-regulation of progestin receptors by P itself. Female hamsters (DeBold, et al., 1976), rats (Gilchrist & Blaustein, 1984), and guinea pigs (Wallen, et al., 1975) that have been rendered sexually receptive by sequential administration of E followed by P usually do not show a further facilitation by a second dose of P. In the guinea pig, this hyposensitivity is accompanied by a reduction of progestin receptors that can only be overcome by a very large dose of P (Blaustein, 1982).

Either of these potential mechanisms could produce a hyposensitivity to P in anestrus animals. Photoperiod-induced differences in P sensitivity have not been

reported; however, differences may be detected when lower doses of P are administered.

Removal of the pineal gland abolishes short-photoperiod induced declines in fertility (Reiter, 1973/74). However, in Experiment 1, knife cuts ventral to the PVN blocked gonadal responses to photoperiod, presumably by disrupting a pathway that provides photic and/or circadian information to the pineal gland, but did not affect photoperiod-induced changes in the activational effects of E on sexual behavior. This finding suggests that the effects of photoperiod on behavioral sensitivity to E are not mediated by the pineal gland. Although both SCN and PVN lesions abolish circadian rhythms in pineal melatonin synthesis (Klein & Moore, 1979; Klein, et al., 1983), the effects of cuts ventral to the PVN on pineal gland functions have not been directly determined. Thus, some effects of photoperiod on the pineal gland may survive cuts that damage SCN-PVN connections. Gonadal and behavioral responses to photoperiod may depend upon different parameters of pineal activity, such as phase relationships between light-dark cycles and melatonin secretion, or amplitude and duration of the melatonin pulse.

In the present study, female hamsters were pinealectomized and OVX and compared with controls in both long and short days in order to determine if photoperiod-induced changes in behavioral sensitivity to E are modulated by the pineal gland. In addition, these animals received varying doses of P in conjunction with constant but low levels of E to test for effects of photoperiod on behavioral sensitivity to P.

Methods

Animals and Housing

Adult female hamsters (LVG/LAK) weighing 110-120 g were maintained under conditions identical to those described in Experiment 1. Only females that showed at least three consecutive 4-day estrous cycles were used in the experiment. Body weights (nearest g) were recorded before each surgery and at the end of the experiment.

Pinealectomies

One group of hamsters (n=21) was anesthetized with Equithesin (4.0 ml/kg) between 0700 and 1300 hr on the day of proestrus and placed in a stereotaxic apparatus (Kopf Instruments). A circular flap of skull (4 mm diameter) with lambda as the midpoint was removed, the superior sagittal sinus reflected, and the pineal body removed from between the cerebral hemispheres with microforceps. Another group of females (n=15) received a sham surgical procedure identical to that of the pinealectomy group except that the pineal gland was not removed. A third group (n=18) was unoperated.

Following surgery, the pinealectomized (PNX) animals and approximately half of the animals in the two control groups (sham-operated, n=7; unoperated, n=11) were transferred to a short-day (6L:18D, lights out at 1800 hr) photoperiod. The remaining control animals were left in the original long-day photoperiod. All animals remained in the assigned photoperiods for the duration of the experiment.

Estrous Cyclicity and Ovariectomy

As a bioassay for successful pinealectomies, the vaginal discharge of each female was inspected daily until all control animals in 6L:18D failed to show estrous cycles for at least two weeks. Only those females in the PNX group that continued to show regular 4-day estrous cycles (n=18, 86%) were used in the behavioral testing. Following the tenth week of data collection on cyclicity, all animals were bilaterally

OVX under Equithesin anesthesia, returned to the respective photoperiods, and allowed to recover for 2 weeks.

Sexual Behavior Testing

Following the recovery period, all animals received a 10 mm silastic capsule (DowCorning; 3.175 mm o.d., 1.575 mm i.d.) containing a crystalline mixture of 25% E diluted with cholesterol. The capsules were implanted subcutaneously under Metofane (methoxyflurane; Pittman-Moore Co.) anesthesia. This dose of E has been shown to induce lordosis in approximately 70% of pineal-intact animals maintained in long days, but only in 25% of animals kept in short days (see Experiment 2a). The females were then tested according to the procedure described in Experiment 2a. All attacks were initiated by the females and in every case, the display of lordosis preceded mounting attempts by the males.

The animals were tested after 3 (Ex3) and 7 (Ex7) days of E treatment. On the eighth day of treatment, all animals received a subcutaneous injection of 250 μg of P in oil 4 hr prior to testing (Ex8 + 250 P). On the following day, the capsules were removed and one week allowed for clearance of E from the animals' systems. The females were then reimplanted for 3 days and received a subcutaneous injection of 10 μg of P 4 hr prior to testing (Ex3 + 10 P). The capsules were removed the following day and reimplanted one week later. All animals were then injected with 20 μg of P 4 hrs prior to testing after 3 (Ex3 + 20 P) and 7 (Ex7 + 20 P) days of exposure to the E implants.

Statistics

Initial between-group comparisons for the percentage of animals responding under each hormone treatment were made using $\underline{X}^2$ analyses. Follow-up tests of selected comparisons were made using Fisher's exact probability tests. For all conditions and for both behavioral measures, latency scores were computed only for animals that

responded during the 5-min. tests. Latency and body weight data were evaluated using one-way analyses of variance (ANOVA), followed by Dunnett *t* tests.

Results

Within photoperiod assignment, comparisons of the percentage of animals showing either lordosis or aggression did not differ between the sham-operated and unoperated control groups for any hormone treatment; thus, these groups were combined for all further statistical analyses. Figure 6 shows the percentage of animals from each group that showed lordosis for each of the six testing conditions. Overall differences in responding across groups were seen for only two of the hormonal conditions; the Ex7 treatment ($p < 0.05$) and the Ex7 + 20 P treatment ($p < 0.02$). Follow-up analyses revealed that for both the Ex7 and Ex7 + 20 P tests, control animals housed in long days were more sensitive to the activational effects of the hormones for the display of lordosis than the control animals housed in short days ($p =$ 0.03 and 0.006 respectively). Also, significantly more control animals housed in long days showed lordosis in response to the two hormone treatments than PNX animals kept in short days ($p = 0.05$ and 0.03).

Analyses of latencies to show lordosis found significant differences among groups only for the Ex7 + 20 P condition (F (2,20) = 3.65, $p < 0.05$; see Table 5). Follow-up comparisons revealed that this difference was due primarily to shorter latencies shown by animals in the long-day control group when compared to the PNX group (t (18) = 2.52, $p < 0.05$).

Figure 7 shows the percentage of animals displaying aggressive behavior at each of the six testing times. The number of animals in each group showing aggressive behavior differed significantly for the Ex3 + 10 P treatment ($p < 0.05$). For that hormonal condition, significantly more control animals housed in short days showed aggressive behavior than did control animals housed in long days ($p = 0.03$) or PNX animals housed in short days ($p = 0.01$). However, none of the comparisons of responded during the 5-min. tests. Latency and body weight data were evaluated using one-way analyses of variance (ANOVA).

Within

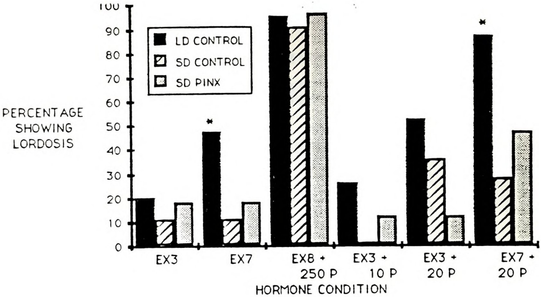

Figure 6. Percentage of animals from each group showing lordosis for each of the hormone conditions in Experiment 2b. *Significantly different from all other groups for these hormonal condition (Ex7: $p < 0.05$; Ex7 + 20 P: $p < 0.02$).

Table 5

Mean (± SEM) latency (sec) to show lordosis and number of animals responding for each treatment group for the six testing conditions in Experiment 2b

	Testing Condition					
	Ex3	Ex7	Ex8 + 250 P	Ex3+ 10 P	Ex3 + 20P	Ex7 + 20 P[a]
				16L:8D Saline		
n =	15	15	15	13	12	12
# Resp. =	3	7	14	3	6	10
Latency =	43.0 (4.2)	97.6 (33.0)	24.1 (5.0)	97.0 (47.2)	77.8 (20.1)	71.4 (15.9)
				6L:18D Saline		
n =	18	18	18	12	12	12
# Resp. =	2	2	16	0	4	3
Latency =	37.5 (0.5)	85.0 (25.0)	51.4 (16.1)	___ ___	106.8 (62.9)	134.3 (47.0)
				6L:18D PINX		
n =	18	18	18	18	18	18
# Resp. =	3	3	17	2	2	8
Latency =	57.3 (12.9)	38.0 (4.6)	42.5 (12.0)	125.0 (25.0)	57.5 (2.5)	137.1[b] (18.2)

[a] Significant overall difference for latency scores ($p < 0.05$).

[b] Significantly different from 16L:8D saline group ($p < 0.05$).

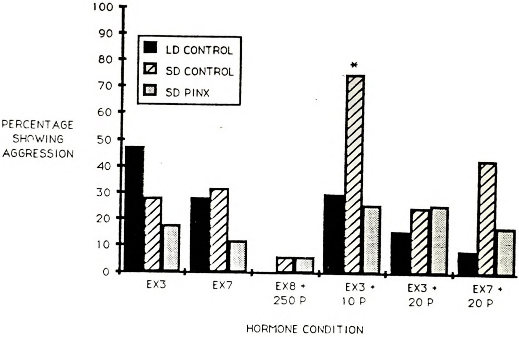

Figure 7. Percentage of animals in each group showing aggressive behavior for each of the hormone conditions in Experiment 2b. *Significantly different from all other groups for this hormonal condition ($p < 0.05$)

Table 6

Mean (± SEM) latency (sec) to show aggression and number of animals responding for each treatment group for the six testing conditions in Experiment 2b

	Testing Condition					
	Ex3	Ex7	Ex8 + 250 P	Ex3 + 10P	Ex3 + 20 P	Ex7 + 20P
			16L:8D Saline			
n =	15	15	15	13	12	12
# Resp. =	7	4	0	4	2	1
Latency =	218.4 (23.0)	174.3 (19.4)	—	151.5 (15.3)	136.5 (68.5)	214.0 (0)
			6L:18D Saline			
n =	18	18	18	12	12	1
# Resp. =	5	6	1	9	3	5
Latency =	131.6 (30.5)	169.5 (29.3)	89.0 (0)	160.4 (24.4)	213.0 (47.0)	178.2 (35.5)
			6L:18D PINX			
n =	18	18	18	18	18	18
# Resp . =	3	2	1	5	5	3
Latency =	244.0 (42.2)	205.0 (70.0)	117.02 (0)	10.0 (38.2)	227.8 (13.4)	156.7 (60.3)

* Numbers in parentheses represent standard error of the mean for latency scores

Table 7

Mean (± SEM) body weights (g) for each treatment group at selected sampling times in Experiment 2b

Group		Sampling Time	
	Pre-surgical	Ovariectomy	Final [a]
16L:8D Saline			
n=	15	15	12
	115.5 ± 2.7	151.7 ± 4.6	145.7 ± 5.4
6L:18D Saline			
n=	18	18	12
	114.4 ± 2.2	165.0 ± 4.4	150.8 ± 3.9
6L:18 PINX			
n=	18	18	18
	113.1 ± 2.4	147.33 ± 4.7	141.8 ± 4.7

[a] 9 control animals died before the end of the experiment.

latency to show aggression resulted in significant differences among groups (see Table 6). Comparisons of body weight among the three treatment groups did not detect significant differences at any sampling point (see Table 7).

Discussion

Short-day induced decreases in sensitivity to the activational effects of exogenous E in inducing female sexual receptivity appear to be independent of the pineal gland. PNX females kept in short days did not show more lordosis behavior in response to E alone than did control animals housed in short days. This finding is in contrast to the effects of pinealectomy on photoperiod-dependent reproductive behaviors reported for male hamsters (Miernicki, et al., 1988). In males, pinealectomy abolishes the decreased sensitivity to T for reinstating copulatory behavior that is induced by exposure to short days. In the present experiment, pinealectomy itself did not interfere with the ability of these animals to display lordosis, as evidenced by the high level of responding when E was supplemented with a high dose (250 μg) of P. However, PNX animals, as well as control animals kept in short days, did not respond to E supplemented with a low dose of P (20 μg) with levels of lordosis as high as those displayed by control animals maintained in long days. Thus, the effects of photoperiod previously reported for E-induced lordosis may be evident also when both ovarian hormones, albeit in low doses, are used to facilitate female sexual receptivity. Since the effects of P on lordosis depend upon the priming effects of E (i.e., induction of neural progestin receptors; Clark, et al., 1982), the reduced effectiveness of E + P treatment reported here may be a special case of a general refractoriness to the effects of E.

While PNX females kept in short days did not show levels of lordosis as high as control animals kept in long days in response to E and a low dose of P, they showed less aggressive behavior when treated with E and a low dose of P (Ex7 + 20 P) than did control animals kept in short days. Thus, it is possible that photoperiodic

modulation of the effects of E and P on lordosis and aggression depend upon different mechanisms for their expression. When maintained in long days, treatment with P alone reduces aggressive behavior in OVX and intact female hamsters (Fraile, et al., 1987a). Possible photoperiodic modulation of this behavioral effect of P has not been investigated. The testing paradigm used in studies of P inhibition of aggression (Fraile, et al., 1987a; Fraile, et al., 1987b) provides an avenue for future study of the effects of day length on P influences on behavior that do not depend upon previous E treatment.

EXPERIMENT 2c

Behavioral Sensitivity to Ovarian Hormones:
Role of Melatonin

Pineal-independent effects of short photoperiods have been reported for prolactin synthesis in female hamsters (Blask, et al., 1986), and exposure to short days can induce increases in body weight and carcass lipid content in female hamsters (Bartness & Wade, 1984) that also appear to be independent of the pineal gland. However, although short-day induced changes in body weight occur even in PNX hamsters, the effects of short-day exposure can still be mimicked by daily afternoon injections of melatonin (M) in animals maintained in long days (see Bartness & Wade, 1985 for a review). This finding suggests that although pineal melatonin may be sufficient to induce photoperiodic effects on body weight, it's presence is not necessary for the effects of short-day exposure to occur. It is possible that dual mechanisms also modulate behavioral sensitivity to hormones. Thus, the following study was designed to determine if the pineal-independent effect of short-day exposure on behavioral sensitivity to exogenous hormones suggested by Experiment 2b is also independent of the effects of daily administration of M.

Methods

Subjects and Housing

Female hamsters (100-110 g) were obtained from Charles River Breeding Laboratory and were maintained under housing conditions identical to those described in Experiment 1. One group of animals (n=8) was housed in a short-day (6L:18D; lights out at 1800 hr) photoperiod. The remaining animals were kept in a long-day (16L:8D; lights out at 1800 hr) photoperiod. Vaginal discharges were monitored daily as in the previous experiments and body weights were recorded at the beginning of the experiment, before OVX, and at the end of behavioral testing.

Injection Procedure

One group of animals (n=15) housed in 16L:8D received daily subcutaneous injections of 10 μg of M (Sigma Chemical Co.) in physiological saline (0.87%) 3 hr prior to lights out. The M solution was prepared weekly from a frozen stock and was kept refrigerated and wrapped in aluminum foil. The remaining hamsters in 16L:8D (n=9) and the control hamsters in 6L:18D (n=8) received daily subcutaneous injections of 0.1 ml of physiological saline 3 hr prior to lights out. Estrous cycles were monitored until all control females in 6L:18D failed to show estrous cycles for at least 2 weeks (10 weeks after transfer to short days). In the M group, only animals that were acyclic at this time (n=8, 53%) were used in the behavioral testing. All animals were then bilaterally OVX under Equithesin anesthesia and returned to their respective photoperiods. Treatment with M and the saline injections continued until the end of the experiment.

Sexual Behavior Testing

After a 2 week recovery period, all animals were implanted subcutaneously with a silastic capsule containing 25% E diluted with cholesterol. All animals were tested for lordosis behavior with sexually active stimulus males (150-160 g) after 7 (Ex7) and 11 (Ex11) days of exposure to E. On the 12th day of implantation, all animals received a

subcutaneous injection of 20 µg of P in oil 4 hr prior to testing (Ex12 + 20 P). The stimulus-male housing and the testing procedures were the same as those described in Experiment 2a and 2b.

Statistics

Statistical analyses were identical to those described for Experiment 2b.

Results

Figure 8 shows the percentage of animals in each treatment group that showed lordosis at each of the three testing times. Animals in the M group showed levels of lordosis in response to E alone that were not significantly higher than saline-treated control animals kept in long days. However, neither of the two groups kept in long days showed levels of lordosis significantly higher than saline-treated control animals kept in short days, and responsiveness of all groups at each testing time was depressed as compared to the percentage of control animals in each photoperiod showing lordosis from previous experiments (see Experiments 2a and 2b). Although the number of animals showing lordosis did not differ at any testing time, latency to show this behavior differed among groups for the Ex12 + 20 P test ($\underline{p} < 0.02$; see Table 8). Saline-treated animals housed in short days showed a mean latency to show lordosis (93.8 ± 33.3 sec) that was significantly longer than those displayed by either animals in the M group (23.7 ± 8.6 sec) or the saline-treated control group in long days (35.5 ± 7.6 sec; $\underline{p} < 0.05$ for both comparisons).

Figure 9 shows the percentage of animals in each group that showed aggressive behavior for each of the three testing conditions. Overall, the 3 treatment groups differed significantly in the number of animals showing aggression only for the Ex11 condition ($\underline{p} < 0.05$). The saline-treated animals housed in short days showed more aggression than either the M group ($\underline{p} = 0.05$) or the saline-treated group ($\underline{p} = 0.04$) kept in long days. Latency to show aggressive behavior did not differ among groups

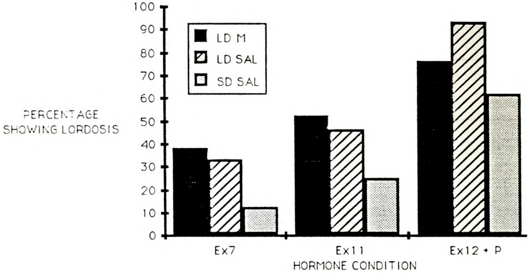

Figure 8. Percentage of animals in each group showing lordosis for each of the steroid treatments in Experiment 2c.

Table 8

Mean (± SEM) latency (sec) to show lordosis and number of animals responding for each treatment group for the three testing conditions in Experiment 2c

Treatment Group	n	Testing Condition Ex7	Ex11	Ex12 + 20 P[a]
16L:8D M	8			
# Resp. =		3	4	6
Latency =		155.3 ± 68.3	61.8 ± 25.5	23.7 ± 8.6
16L:8D Saline	9			
# Resp. =		3	4	8
Latency =		130.3 ± 45.9	114.8 ± 39.3	35.5 ± 7.6
6L:18D Saline	8			
# Resp. =		1	2	5
Latency =		47.0	17.0 ± 8.0	93.8 ± 33.3[b]

[a] Significant overall difference among groups for latency scores, ($p < 0.02$).

[b] Significantly different from 16L:8D M ($p < 0.05$) and from 16L:8D saline ($p < 0.05$).

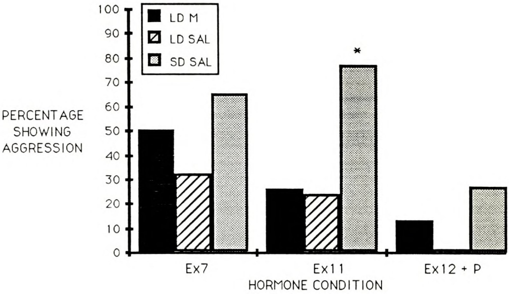

Figure 9. Percentage of animals in each group showing aggressive behavior for each steroid treatment in Experiment 2c. *Significantly different from all other groups for that testing condition ($\underline{p} < 0.05$).

Table 9

Mean (± SEM) latency (sec) to show aggressive behavior and number of animals responding for each treatment group at the three testing conditions in Experiment 2c

Treatment Group	n	Testing Condition Ex7	Ex11	Ex12 + 20 P
16L:8D M	8			
# Resp. =		4	2	1
Latency =		145.0 ± 22.9	205.5 ± 71.5	209.0
16L:8D Saline	9			
# Resp. =		3	2	0
Latency =		177.7 ± 41.7	269.0 ± 7.0	____
6L:18D Saline	8			
# Resp. =		5	6	2
Latency =		110.6 ± 29.1	195.3 ± 25.2	160.0 ± 113.0

Table 10

Mean (± SEM) body weights (g) for each treatment group at selected sampling times in Experiment 2c

Treatment Group	n	Sampling Time Initial	Ovariectomy	Final
16L:8D M	8	30.5 ± 3.5	161.5 ± 2.9	144.9 ± 4.8
16L:8D Saline	9	130.4 ± 4.0	145.3 ± 7.2	146.0 ± 6.8
6L:18D Saline	8	133.6 ± 3.6	156.1 ± 5.7	136.0 ± 4.6

for any hormonal condition (see Table 9). Body weight did not differ significantly among groups at any sampling point (see Table 10).

Discussion

In general, the display of lordosis or aggression in the M-treated animals tended to parallel that displayed by saline-treated animals kept in long days. However, except for latency to show lordosis in response to E in combination with a low dose of P (Ex12 + 20 P), and the number of animals showing aggression following exposure to E alone (Ex11), the groups housed in long days did not differ significantly in the display of hormone-dependent sociosexual behaviors from animals housed in short days. This failure to obtain a more robust effect of photoperiod is in contrast to previous observations (Experiments 2a and 2b), and may be due to the small sample size and overall refractoriness to steroid hormones seen in the animals from this experiment. The present results do suggest, however, that M treatment does not equally affect all aspects of female reproductive physiology and behavior. Although M administration mimicked the effects of short days on estrous cyclicity, the expression of hormone-dependent sociosexual behaviors by M-treated hamsters more closely resembled the pattern displayed by control animals kept in long days. In a previous experiment (Morin, 1982), a higher dose of M (25 μg/day) administered for 4 days or 6 weeks also failed to affect the display of lordosis in hamsters treated with EB and P.

Experiments using Siberian hamsters have shown that the duration of the nocturnal M peak is the critical parameter in the transduction of photoperiodic information (see Underwood & Goldman, 1987, for a review). However, for Syrian hamsters, the characteristics (i.e., duration, amplitude) of the pattern of nocturnal M secretion that are involved in the mediation of reproductive responses are not fully understood. It is

possible that physiological and behavioral responses to photoperiod rely upon different parameters of the M rhythm for their expression. Thus, the regime of exogenous M administration employed in the present study may have interacted with the animals' endogenous rhythm to provide a signal sufficient to induce estrous acyclicity, but not decreased behavioral sensitivity to ovarian hormones. Further studies employing M infusion techniques that may more closely mimic physiological patterns of secretion under different photoperiods (Bartness & Goldman, 1986) should serve to elucidate the role of this hormone in the photoperiodic control of reproductive physiology and behavior.

In summary, the claim for a negligible role of the pineal gland in the modulation of photoperiodic effects on behavior proposed in Experiment 2b is further supported by the apparent inability of M to mimic short-day induced patterns of sociosexual behavior in the present study. The role of the pineal gland as a transducer of daylength information into an endocrine signal is well established (see Elliott & Goldman, 1981, for a review). However, photoperiod may also directly influence steroid-sensitive neural systems responsible for female sexual behavior. The direct retinal input to the SCN of mammals appears to be sufficient for the entrainment of circadian rhythms to the light-dark cycle (Klein & Moore, 1979; Moore & Klein, 1974), and is probably necessary for the pineal-dependent responses to changes in daylength. However, anatomical studies of the retinal projections of the hamster (Pickard & Silverman, 1981; Youngstrom, et al., 1987) have identified additional direct inputs to basal forebrain and hypothalamic areas outside the SCN. Some of these regions concentrate ovarian steroids (Fraile, et al., 1987a; Morrell & Pfaff, 1978) and may play a role in the control of female sociosexual behavior. Thus, it is possible that hypothalamic and basal forebrain retinal inputs outside the SCN affect steroid sensitivity independently of the pineal gland.

EXPERIMENT 3

Effects of Knife Cuts Dorsal to the PVN on Reproductive Responses to Photoperiod

Lesions of the PVN abolish gonadal responses to photoperiod in male and female hamsters (Bartness, et al., 1985; Lehman, et al., 1984; Pickard & Turek, 1983). In females, the path taken by PVN projections involved in gonadal responses to photoperiod is unknown. In rats, two sets of projections to the spinal cord exit the PVN; one in a dorsal and one in a lateral direction (Luiten, et al., 1985). In hamsters, knife cuts dorsal to the PVN interrupt fibers that appear to be important in the mediation of gonadal responses to photoperiod in males (Inouye & Turek, 1986; Nunez, et al., 1985). In contrast, similar dorsal knife cuts in four female hamsters from Experiment 1 failed to prevent short-day induced estrous acyclicty and uterine regression. However, all of the females with knife cuts dorsal to the PVN were housed in short days. Thus, it is not certain whether the failure of these cuts to block gonadal responses to photoperiod reflects a sex difference in the functional neuroanatomy of PVN efferent projections, or whether these cuts instead disrupt a pathway that is necessary for the expression of estrous cyclicity independent of photoperiod. In order to further investigate the function of PVN projections to the spinal cord, knife cuts dorsal to PVN were examined in this study for their role in photoperiod-dependent estrous cyclicity.

Methods

Subjects and Housing

Adult female golden hamsters were obtained and housed under conditions identical to those described in Experiment 1. Only females that showed at least three consecutive 4-day estrous cycles were used in the experiment.

Surgery

The hamsters were anesthesized and received knife cuts (n=18) or sham-surgery (n=10) using a procedure similar to that outlined in Experiment 1. Stereotaxic coordinates for the knife cuts were 0.3 mm posterior to the bregma, 5.9 mm ventral to the dura, with the top of the incisor bar 2.0 mm below ear bar zero.

Estrous Cyclicity

Following surgery, the hamsters were returned to the long-day photoperiod and were permitted to recover for one week. Twelve hamsters with knife cuts and five of the sham-operated control animals were then transferred to a short-day photoperiod. Vaginal discharges were inspected daily until all sham-operated animals in the short-day photoperiod failed to show an estrous discharge for at least 2 weeks. At the completion of data collection on cyclicity, the animals were anesthesized and the uteri photographed as in Experiment 1. Uterine width measurements to the nearest 0.1 mm from the left uterine horn were taken from the photographs by two raters unaware of the animals' group assignments. A correlation of the two rater's scores revealed a high degree of agreement ($\underline{r} = 0.97$). An average score was used in those cases where the measurements differed. The uteri were then removed and weighed to the nearest 0.1 mg.

Histology

Perfusions and histological preparation of the neural tissue was the same as outlined in Experiment 1. Microscopic examination was used to verify the extent and location of knife cuts. Schematic diagrams were made to illustrate the extent and location of the knife cuts with respect to the PVN. The evaluation of the knife cut placements was performed without knowledge of the effects of surgery on gonadal responses to photoperiod.

Statistics

Statistical analyses of body weight, uterine weight, and uterine width were conducted as described for Experiment 1.

Results

Placement of Knife Cuts

Figure 10 shows the glial scar resulting from the knife cut in a representative animal from the 6L:18D photoperiod (No. 5). In all except 2 of the animals kept in 6L:18D, the knife cuts in animals in both photoperiods were bilateral and confined to an area just dorsal to (16L:8D: n=5; 6L:18D: n=7) or through the dorsal one third (16L:8D: n=1; 6L:18D: n=3) of the PVN. Exceptions to the dorsal knife cut placement were one animal that had a bilateral cut which passed through the ventral portion of the PVN, and one other animal that had a bilateral but asymmetrical cut through the most ventral portion of the PVN. As previously reported (Badura, et al., 1987a), cuts in these locations prevented short day photoperiod-induced acyclicity and uterine regression. Mean uterine width of these hamsters was 3.23 + 0.47 mm and mean uterine weight was 0.53 + 0.06 g. These two animals were not included further in the analyses. Figure 11 depicts schematically the location of the center of the knife cuts for the remaining animals in both photoperiods.

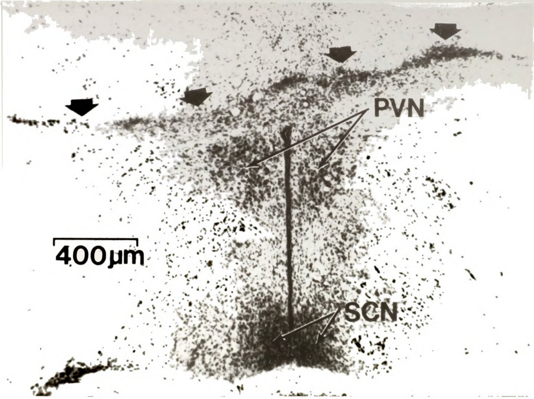

Figure 10. Photomicrograph of an oblique coronal section (40 µm thick; cresylecht violet stain) of a representative hamster (No. 5) with a knife cut just dorsal to the paraventricular nucleus from the 6L:18D photoperiod. The knife cut (indicated by arrows) failed to prevent gonadal responses to photoperiod after 10 weeks of exposure to short days.

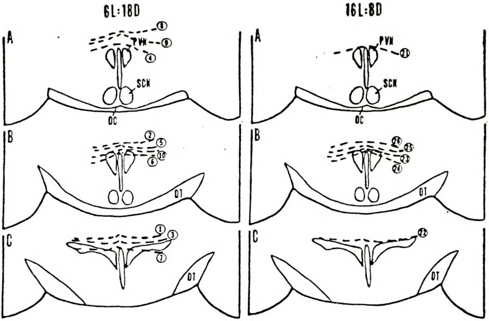
6L:18D
16L:8D
A
B
C
PVN
SCN
OC
OT
⑧
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Figure 11. Schematic representation of the location of knife cuts that produced bilateral damage dorsal to the paraventricular nucleus (PVN; n=12) or through the dorsal one third of the PVN (n=4) of female hamsters kept in either 16L:8D or 6L:18D photoperiods. The drawings of the hamster hypothalamus were modified from Lehman, et al., (1984) and the individual cases are identified by numbers (see Figure 2 for abbreviations).

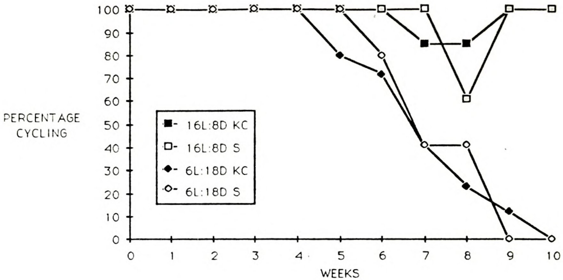

Figure 12. Percentage of animals with knife cuts (KC) or sham-operated animals (S) showing normal 4-day estrous cycles during each week of exposure to 16L:8D or 6L:18D photoperiods (16L:8D KC, n=6; 6L:18D KC, n=10;16L:8D S, n=5; 6L:18D S, n=5).

Table 11

Mean (± SEM) uterine weight (g) and uterine width (mm) for each group in Experiment 3

Group	n	Uterine Weight (g)	Uterine Width (mm)
16L:8D KC	6	0.58 ± 0.05	3.43 ± 0.24
16L:8D S	5	0.44 ± 0.05	3.49 ± 0.25
6L:18D KC	10	0.20 ± 0.01*	1.83 ± 0.14
6L:18D S	5	0.18 ± 0.05	1.90 ± 0.17

* This measure was computed for 7 of the 10 animals.

Cyclicity and Uterine Condition:

All groups of females continued to show 4-day estrous cycles during the one-week postoperative recovery period in long days. Figure 12 shows the percentage of animals in each group showing estrous cycles for the 10 weeks of exposure to the 16L:8D or 6L:18D photoperiods. Animals with knife cuts dorsal to or through the dorsal one third of the PVN and sham-operated control hamsters that remained in the 16L:8D photoperiod continued to cycle throughout the data collection period (see Figure 12), with the exception of 2 sham-operated animals that became spontaneously acyclic during week 8, and two animals with knife cuts that each became acyclic for one week (No. 21 during week 7 and No. 25 during week 8). All of these animals maintained stimulated uteri (see Table 11). In contrast, animals with knife cuts dorsal to or through the dorsal portions of the PVN and sham-operated animals in the 6L:18D photoperiod were acyclic within 9 weeks of transfer from long days. These animals had uteri that were significantly regressed as compared with animals in the long day photoperiod (Table 11).

For all animals, there was a significant positive correlation between uterine width and uterine weight scores ($\underline{r} = 0.91$, $p < 0.01$). In addition, the average uterine width of animals with knife cuts and sham-operated animals maintained in 6L:18D was significantly smaller than for either group of animals housed in 16L:8D ($\underline{F}$ (1,22) = 73.14, $\underline{p} < 0.001$; see Table 11). However, there was no effect of surgery on uterine width within the two photoperiod groups ($\underline{F}$ (1,22) = 0.13, $p > 0.05$). There was a significant effect of photoperiod on uterine weight, with animals in 6L:18D having lower weight values than animals in 16L:8D ($\underline{F}$ (1,19) = 77.10, $\underline{p} < 0.001$). Unlike uterine width measurements, there was also an effect of surgical condition on uterine weight ($\underline{F}$ (1,19) = 4.94, $\underline{p} < 0.05$). Follow-up comparisons revealed this effect was due to significantly higher uterine weights for animals with knife cuts than for sham-operated control animals in 16L:8D ($\underline{t}$ (19) = -2.51, $\underline{p} < 0.05$).

Discussion

In rats, the PVN send two separate projections to the spinal cord (Luiten, et al., 1985). One set of fibers (bundle 1) takes a dorso-caudal route and the other (bundle 2) courses laterally upon leaving the nucleus. Recently, a similar anatomical organization of PVN projections has been described in hamsters (Youngstrom & Nunez, 1986). Therefore, in male hamsters, damage to bundle 1 produced by knife cuts dorsal to the PVN may be sufficient to prevent photoperiodic responses of the reproductive system. However, recent evidence from our laboratory (Youngstrom & Nunez, 1988) indicates that the dorsal efferent projection in hamsters is very sparse and travels in a caudal direction just above the third ventricle. In males, knife cuts that appear to be located in a region dorsal to the PVN that would not disturb these fibers still prevent testicular regression in short days (Inouye & Turek, 1986; Nunez, et al., 1985). Thus, it is not yet certain whether this sparse dorsal projection is significantly involved in the transfer of photic and circadian information to the pineal gland.

In contrast, knife cuts dorsal to or through the dorsal one third of the PVN of females in the present study did not prevent short-photoperiod induced acyclicity and uterine regression. Thus, in females, dorsal efferent projections of the PVN (bundle 1) are not necessary for the display of photoperiodic responses. The fact that these cuts did not induce acyclicity in animals maintained in long days further indicates that dorsal projections are not necessary for the display of estrous cycles. These results contrast with the findings that knife cuts located ventral to or through the ventral portions of the PVN do abolish reproductive responses to photoperiod in female hamsters (Experiment 1), and that knife cuts either ventral or dorsal to the PVN block short day-induced testicular regression in males (Eskes & Rusak, 1985; Inouye & Turek, 1986; Nunez, et al., 1985). The differential effects of knife cuts placed dorsal to the PVN in males and females suggests the possibility of a sex difference in the functional anatomy of the hypothalamic circuits involved in the modulation of gonadal responses to photoperiod

in hamsters. In contrast to the anatomical findings for bundle 1, bundle 2 in the hamster appears to contain a massive plexus of efferent fibers (Youngstrom & Nunez, 1988). Parasagittal knife cuts located lateral to the PVN that would disrupt PVN laterally projecting efferent fibers prevent short-photoperiod induced estrous acyclicity in female hamsters (Kelly, et al., 1988). This finding, taken in consideration with the results of Experiment 1 and the present study, suggests that damage to bundle 2 may be necessary to prevent gonadal responses to photoperiod in females.

Gonadal responses to photoperiod in male hamsters can be prevented by olfactory bulbectomy (Clancy, et al., 1986; Pieper, et al., 1984). Bulbectomized animals maintain large testes in short days and show significant elevations in circulating levels of FSH in both long and short photoperiods (Clancy, et al., 1986). Similarly, knife cuts located dorsal to the PVN in male hamsters exaggerate the rise in FSH levels that occurs following castration independently of photoperiod (Badura, et al., 1988). It is possible that both olfactory bulbectomy and knife cuts dorsal to the PVN disrupt hypothalamic control of gonadotropin secretion. High levels of FSH would contribute to the maintainence of large testes in male hamsters exposed to short days. Females, on the other hand, require synchronized changes in circulating levels of both FSH and LH to display estrous cycles, and elevated FSH levels alone would not provide sufficient stimulation to allow estrous cycles to continue in short days. Gonadotropins were not measured in the present study, and thus it is not known whether the knife cuts affected FSH or LH levels independently of photoperiod. The possibility exists that knife cuts ventral and dorsal to the PVN function via separate mechanisms, i.e., ventral cuts by interrupting the transfer of photic information to the pineal gland and dorsal cuts by interfering with gonadotropin secretion. It is also possible that cuts dorsal and ventral to the PVN differentially disrupt pineal melatonin secretion, and males may be more sensitive to these disruptions.

EXPERIMENT 4

Photoperiodic Effects on Plasma Levels of FSH: Effects of Knife Cuts Ventral to the PVN

Female hamsters maintained in long and short days exhibit markedly different patterns of gonadotropin secretion. Changes in the secretion of LH and progesterone induced by exposure to short days precede the onset of vaginal acyclicity by several weeks (Jorgenson & Swartz, 1985). On the afternoon of vaginal estrus, plasma levels of progesterone are significantly elevated almost immediately following transfer to short days. LH levels also increase, albeit more slowly, with the elevations becoming detectable only after the animals have been in short days for 2-3 weeks. A consistent elevation of FSH does not appear to occur until the animals are acyclic. Despite these abnormalities in the endocrine profile, the animals continue to display 4-day estrous cycles.

The pineal gland has been implicated in the control of a number of photoperiodic effects on gonadotropin secretion. In males kept in short days, pinealectomy results in post-castration rises in LH and FSH that are more similar to those shown by animals kept in long days (Turek, et al., 1983), and administration of melatonin can induce increases in sensitivity to the negative feedback effects of testosterone in animals maintained in long days (Sisk & Turek, 1982). In females housed in long days, daily administration of melatonin induces acyclicity that is accompanied by a pattern of gonadotropin secretion that is characteristic of anestrous animals in short days (Tamarkin, et al., 1976b) In addition, surgical manipulations that induce a disruption of the melatonin rhythm (i.e., superior cervical ganglionectomy, SCN lesions, etc) result in subsequent alterations in LH and FSH secretion (see Tamarkin, et al., 1985, for a review).

In Experiment 1, horizontal knife cuts placed ventral to or through the ventral portions of the PVN blocked short-photoperiod induced acyclicity and uterine regression. Presumably, these knife cuts disrupted the neural regulation of the pineal gland, and hence melatonin secretion. Since gonadotropin levels were not measured in that study, it is not known whether these knife cuts also prevented the effects of short days on the pattern of gonadotropin secretion. Since animals with abnormal gonadotropin profiles can continue to show apparently normal 4-day estrous cycles for several weeks (Jorgenson & Schwartz, 1985), it also remains possible that the knife cuts induced changes in the amplitude and/or temporal pattern of gonadotropin secretion that still permitted the animals to cycle.

In the following study, plasma FSH levels were assessed across a 24-hr period in neurally intact females, as well as females with knife cuts that prevented gonadal regression, in order to investigate potential differences in gonadotropin secretion.

Methods

Subjects and Housing

Cycling female hamsters were initially individually housed under conditions identical to those outlined in Experiment 1. Only females that showed at least 3 regular 4-day estrous cycles prior to surgery were used in the experiment. Body weights in all animals were monitored weekly throughout the experiment.

Surgical Procedure

One group of hamsters (n=18) received knife cuts according to the procedure described in Experiment 1. Coordinates were 6.6 mm ventral to the sinus and 0.3 mm postbregma to a point aimed midway between the PVN and the SCN. Another group of females (n=26) received a sham surgical procedure.

All animals were returned to the 16L:8D photoperiod for 1 week and allowed to recover from surgery. On the morning of proestrus the following week, half of the animals with knife cuts (n=9) and half of the sham-operated animals (n=11) were moved to a nonstimulatory (6L:18D) photoperiod with lights off at 1800 h. All animals remained in the assigned photoperiods for the duration of the experiment.

Estrous Cyclicity

Vaginal discharges were monitored daily in each group until all of the sham-operated animals housed in short days showed discharges characteristic of anestrus for at least 1-2 weeks. At this time, a subset of the animals in each group were prepared for blood sampling.

Cannulation Procedure

24 hr prior to sampling, the selected animals were anesthetized (Equithesin) and the right jugular vein exposed and cleared of connective tissue. A cannula was inserted into the vein through a small incision and positioned at the entrance to the right atrium. The cannula consisted of Silastic tubing (Dow-Corning Co.), with a PE cuff located approximately 30-35 mm from the end, inserted into the jugular vein. The cannula was

secured in the vein with a loop of suture on either side of the PE cuff. The other end of the cannula was directed subcutaneously to emerge from the animal's back midway between the shoulder blades. The tubing was housed in a flexible metal spring attached to a 3-way rotating valve mounted above the cage and was kept filled with heparinized Krebs ringer (HKR).

Donor Blood Preparation

48 hr prior to blood sampling, donor blood was obtained from intact male hamsters via decapitation. The trunk blood was collected into a heparinized citrate-phosphate-dextrose-adenine mixture (CPDA) to keep the red blood cells viable and to prevent clotting. The donor blood was filtered into centrifuge bottles and washed and centrifuged twice with Krebs ringer bicarbonate (KRB). The supernatant from centrifuging was aspirated and discarded each time. The red blood cells were then resuspended with an equal volume of 5% human plasma protein fraction (Plasmanate; Cutter Biological). Glucose (0.5 mg/ml), heparin (2 U/ml), and penicillin (100 U.ml; Benzylpennicilin, Sigma Chemical Co.) were also added, and the mixture was stored at 5o C for 48 hr before use.

Blood Sampling Procedure

During sampling, blood was first drawn into a 3 cc syringe containing 0.6-0.8 ml of donor blood attached to the side port of the valve in order to remove HKR from the tubing. The 3 cc syringe was replaced with a 1 cc syringe, and a sample of blood (0.6-0.8 ml) was drawn via the side port. The sample was transferred to a heparinized plastic collection tube, vortexed, and stored on ice prior to centrifuging. The 3 cc syringe containing donor blood was then reattached, a small quantity of blood drawn into the syringe to remove air bubbles, and the contents slowly injected back into the animal. Thus, blood samples were replaced with an equal volume of donor blood. Finally, the cannula was flushed with HKR via a 3 cc syringe attached to the top port of the valve. Each animal was sampled every hour throughout a 24 hr period. Cycling

animals were sampled during proestrus. Within 6-12 hours of blood sampling, the samples were centrifuged and the plasma was removed and frozen until RIA.

Radioimmunoassay Procedure

The plasma was assayed for FSH using the NIAMDD rat FSH kit (National Hormone and Pituitary Program, courtesy of Dr. Raiti) employing NIADDK-FSH RP-2 as the reference standard and iodinated NIADDK-rFSH-I6 as trace (8500 cpm/50 µl). NIADDK-anti-rFSH-S-11 (diluted with 3% NRS at an initial ratio of 1:20,000) was the first antibody and sheep anti-rabbit gamma globulin 1306 (SARGG; Pel-Freez Biologicals, diluted with 0.1% gel-PBS, pH 7.4, at a ratio of 1:15) was used as the second antibody in the double antibody RIA. The NIADDK rat assay was previously validated for use with hamster plasma by Bast & Greenwald, (1974). Results plotted from Quality Control Standard dilution curves (QCS pools; plasma samples run at various dilutions) for pools of intact male hamsters (medium FSH), castrated male hamsters (high FSH), and castrated males implanted with a 4 mm T capsule (low FSH) were used to determine the plasma sample volume of 65 µl for use in this assay. Samples were run in duplicate and FSH values were expressed in terms of ng equivalents of NIADDK-rFSH-RP-2 per ml of plasma. Each reagent, except secondary antibody, was incubated for 24 hr at 4 o C. SARGG 1306 was incubated for 48 hr. Following incubation, all tubes were spun in a Sorvall refrigerated centrifuge, decanted, and the radioactivity of the precipitate counted with an automatic gamma counter. The lower limit of detectability (90%) of the assay was 7.6 ng/ml and the intraassay coefficients of variance for the high, medium, and low QCS pools were 6.2%, 11.6%, and17.5 % respectively.

Uterine Weight and Histological Procedures

Following blood sampling, the animals were laparotomized and the uteri removed and weighed (nearest 0.1 mg). Animals with knife cuts were sacrificed, perfused, and the brains prepared for histological examination as described in Experiment 1.

Statistics

Between group comparisons for body weight and uterine weight were conducted as outlined for Experiment 1. The FSH data from individual animals were first analyzed using the PC Pulsar program of Merriam & Wachter (1982) adapted for use on the IBM PC (Gitzen & Ramirez, 1986). This program provides an algorithmic analyses using a set of criteria (G values) that are based upon the intraassay coefficient of variation (cv) and are standardized across all cases to detect and characterize surges in individual animals. The G values represent the number of cv's that one, two, three, four, or five data points must be over a smoothed baseline in order to be characterized as a pulse. In the present analysis, G values were: G(1) = 3.2, G(2) = 2.8, G(3) = 2.3, G(4) = 1.9, G(5) = 1.5. The resulting data were then analyzed using two-way ANOVAs (photoperiod x surgical condition) to investigate potential differences in FSH secretion across groups.

Results

Placement of Knife Cuts

Figure 13 shows the glial scar resulting from a knife cut located ventral to the PVN in a representative animal (No. 16) from the 16L:8D photoperiod. For animals in 6L:18D, all knife cuts that prevented the effects of short photoperiods on estrous cyclicity and uterine weight were bilateral and located ventral to (7 cases) or through the ventral portions (2 cases) of the PVN. The knife cut in one of these animals (No. 12) also resulted in an area of mechanical damage dorsal to the PVN. Four other animals in 6L:18D had cuts that either spared most or all of the PVN (2 animals), passed through the dorsal most portions of the PVN (1 animal), or were ventral to the PVN but so asymmetrical as to be nearly unilateral (1 animal). These four animals became acyclic within 8 wks of exposure to 6L:18D and were not included further in the analyses.

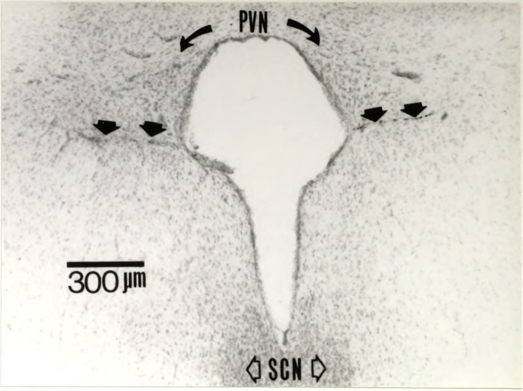

Figure 13. Photomicrograph of an oblique coronal section (40 µm thick; cresylecht violet stain) of a representative hamster (No. 16) with a knife cut just ventral to the paraventricular nucleus from the 6L:18D photoperiod. The knife cut (indicated by arrows) prevented gonadal responses to photoperiod after 10 weeks of exposure to short days.

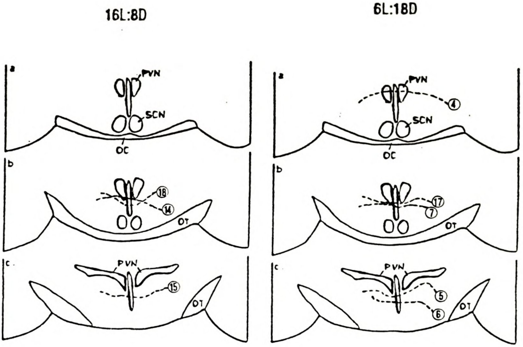
16L:8D
6L:18D
a
PVN
SCN
OC
b
⑱
⑭
OT
c
PVN
⑮
OT
a
PVN
④
SCN
OC
b
⑰
⑦
OT
c
PVN
⑤
⑥
OT

Figure 14. Schematic representation of the location of knife cuts that produced bilateral damage ventral to the paraventricular nucleus (PVN; n=13) or through the ventral two thirds of the PVN (n=5) of female hamsters kept in either 16L:8D or 6L:18D photoperiods. The drawings of the hamster hypothalamus were modified from Lehman, et al., (1984) and the individual cases are identified by numbers (see Figure 2 for abbreviations).

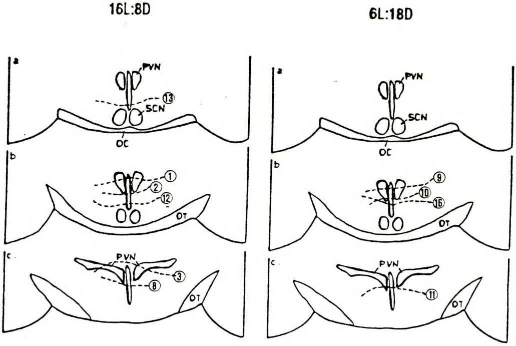
16L:8D
6L:18D
a
PVN
⑬
SCN
OC
b
①
②
⑫
OT
c
PVN
③
⑧
OT
a
PVN
SCN
OC
b
⑨
⑩
⑯
OT
c
PVN
⑪
OT

Figure 14 (cont.)

Similarly, all knife cuts in animals in 16L:8D, except one, were bilateral and located ventral to (6 cases) or through (3 cases) the PVN. One animal had a knife cut that was centered in the VMH area, completely sparing the PVN. This animal was not included further in the analyses. The locations of the area of the greatest extent of damage caused by the knife cuts ventral to or through the PVN for animals in both 16L:8D (n=9) and 6L:18D (n=9) are depicted schematically in Figure 14.

Estrous Cyclicity

In both photoperiods, all animals with knife cuts ventral to or through the ventral portions of the PVN continued to show regular 4-day estrous cycles throughout the experiment. Likewise, except for one animal that became spontaneously acyclic during Week 3, and 2 animals that were acyclic during Week 6, all sham-operated animals in 16L:8D also continued to cycle during the 11 weeks post-surgery. In contrast, all sham-operated animals in 6L:18D showed discharges characteristic of anestrus after exposure to short days for 9 weeks (see Figure 15).

Uterine Weight and Body Weight

An analysis of uterine weight measurements found a significant interaction between photoperiod and surgical group, $F\ (1,40) = 14.85$, $p < 0.005$. There were also significant main effects for both photoperiod ($F(1,40) = 20.22$, $p < 0.001$) and surgical condition ($F(1,40) = 50.57$, $p < 0.001$). Follow-up analyses revealed that animals with knife cuts in 16L:8D had larger uterine weights than sham-operated animals in either 16L:8D ($t\ (22) = 2.40$, $p < 0.05$) or 6L:18D ($t(18) = 7.99$, $p < 0.001$), but were not significantly different from animals with knife cuts in 6L:18D ($p > 0.05$). Animals with knife cuts in 6L:18D had larger uterine weights than their respective sham-operated control group ($t\ (18) = 7.54$, $p < 0.001$), but were not significantly different from either group in 16L:8D ($p > 0.05$ for both comparisons). Finally, sham-operated animals in 16L:8D had larger uterine weights than sham-operated animals in 6L:18D ($t(9)= 6.47$, $p < 0.001$). Thus, within photoperiod,

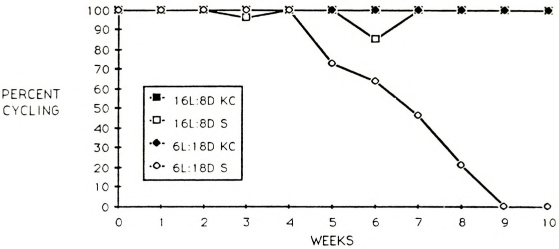

Figure 15. Percentage of animals with knife cuts (KC) or sham-operated animals (S) showing normal 4-day estrous cycles during each week of exposure to 16L:8D or 6L:18D photoperiods (16L:8D KC, n=9; 6L:18D KC, n=9;16L:8D S, n=15; 6L:18D S, n=11).

animals with knife cuts had larger uterine weights than sham-operated animals, and animals in 16L:8D tended to have larger uterine weights than animals in 6L:18D (see Table 12).

Similar results were obtained for uterine weight in the cannulated animals. There was a significant interaction, F (1,14) = 5.41, $p < 0.05$, as well as significant main effects for photoperiod, F (1,14) = 7.07, $p < 0.02$, and surgical condition, F (1,14) = 25.01, $p < 0.002$. Sham-operated animals in 6L:18D had smaller uterine weights (0.266 + 0.014 g) than animals with knife cuts in 16L:8D (0.450 + 0.042 g; t (7) = 9.68, $p < 0.001$) or 6L:18D (0.438 + 0.044; t (7) = 9.05, $p < 0.001$), as well as sham-operated animals in 16L:8D (0.355 + 0.015; t (6) = 4.05, $p < 0.01$). In addition, sham-operated animals in 16L:8D had smaller uterine weights than animals with knife cuts in 16L:8D, t (7) = 5.0, $p < 0.01$, or in 6L:18D, t (7) = 4.37, $p < 0.01$.

Body weight gain, rather than total body weight, was computed in this study to minimize the potentially confounding effects of post-surgical weight loss across groups. The amount of body weight gained post-surgery was determined by subtracting body weight at the time of photoperiod transfer from the final weight (Week 11 post-transfer). An analysis of body weight gain across groups found significant main effects for both photoperiod (F(1,10) = 13.28, $p < 0.01$) and surgical condition (F(1,40) = 12.20, $p < 0.01$). Follow-up analyses revealed that sham-operated animals in 16L:8D gained less weight over the 11 week post-photoperiod transfer period than animals with knife cuts in 16L:8D (t (22) = 3.32, $p < 0.01$), as well as animals with knife cuts (t (22) = 5.23, $p < 0.001$) and sham-operated animals (t (24) = 3.64, $p < 0.01$) in 6L:18D. Animals with knife cuts in 16L:8D did not gain significantly more weight than animals with knife cuts or sham-operated animals in 6L:18D, nor did animals with knife cuts in 6L:18D differ from their respective sham-operated control group ($p > 0.05$ for all comparisons). Thus, both short photoperiod and knife cuts induced larger gains in body weight (see Table 12).

Table 12

Mean (± SEM) uterine weight and body weight gain for animals in Experiment 4

Group	n	Uterine Weight (g)	Body Weight Gain (g)
16L:8D KC	9	0.479 ± 0.04 [a]	54.44 ± 11.73
16L:8D S	15	0.398 ± 0.02	24.07 ± 3.25 [c]
6L:18D KC	9	0.463 ± 0.03	71.89 ± 8.57
6L:18D S	11	0.192 ± 0.01 [b]	55.46 ± 3.70

[a] Significantly different from both sham-operated groups.
[b] Significantly different from all other groups.
[c] Significantly different from all other groups.

FSH Profiles

Plasma FSH levels across the 24 hr sampling period for a representative animal from each group are shown in Figure 16. In general, the PC Pulsar program detected an FSH peak for each animal, except for one animal with a knife cut in 16L:8D (No. 17) and one in 6L:18D (No. 18) in which no peaks were detected. All other animals showed a single peak, except for one sham-operated animal in 6L:18D and one animal with a knife cut in 16L:8D (No. 5) that showed 3 separate peaks, and one additional animal with a knife cut in 16L:8D (No. 16) that had 2 separate peaks. The characteristics of the FSH levels for each of these individual animals are listed in Table 13. For animals with more than one peak, the peak of largest amplitude was chosen for inclusion in subsequent analyses.

Table 14 summarizes the characteristics of FSH secretion across the 24 hr for each group. For comparisons of characteristics involving clock time at which the surge occurred (time of peak onset and time of maximum peak), each hr of the 24 hr sampling period was assigned a value of 1-24 (i.e., 1000 hr = 10 and 1400 hr = 14). No significant differences were found for mean FSH levels computed across the 24 hr, nor were differences found for mean peak duration or the mean values of the maximum peak ($\underline{p} > 0.05$ for all analyses). However, a significant interaction between photoperiod and surgical condition was found for the time of peak onset, $\underline{F}$ (1,10) = 55.72, $\underline{p} < 0.0001$. There were also significant main effects for both photoperiod, $\underline{F}$ (1,10) = 24.26, $\underline{p} < 0.001$, and surgical condition, $\underline{F}$ (1,10) = 23.30, $\underline{p} < 0.001$, for this variable. Follow-up analyses revealed that time of peak onset did not differ significantly among animals with knife cuts in 16L:8D, animals with knife cuts in 6L:18D, and sham-operated animals in 16L:8D ($\underline{p} > 0.05$ for all comparisions), but that each of these groups differed significantly from sham-operated animals in 6L:18D: sham-operated animals in 6L:18D vs animals with knife cuts in 6L:18D, $\underline{t}$ (4) = -3.95,

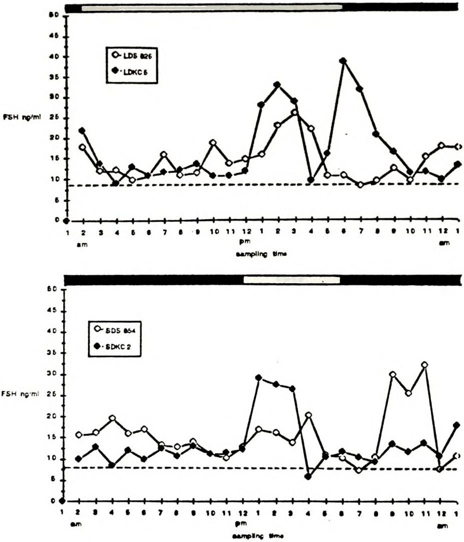

Figure 16. FSH levels across the 24 hr sampling period for a representative animal from each group. (LDKC= No. 5, SDKC = No. 2). Horizontal dotted line denotes the lower limit of detectability of the assay. Black/white bars above each graph denote the light-dark cycle for each photoperiod.

Table 13

Peak values (ng/ml), time of onset, and duration of peaks for animals with more than one peak during the 24 hr sampling period

Animal Group	Maximum Peak Value	Time of Max. Value	Peak Amplitude	Peak Duration	Peak Onset
854	(1) 26.05	1400	14.18	1 hr	1400
SDS	(2) 20.24	1600	8.99	1 hr	1600
	(3) 32.09	2300	22.90	3 hr	2100
5	(1) 21.89	200	9.56	1 hr	200
LDKC	(2) 32.99	1400	21.94	3 hr	1300
	(3) 38.50	1800	27.31	5 hr	1700
16	(1) 21.00	1200	10.17	3 hr	1200
LDKC	(2) 27.35	1700	16.72	2 hr	1600

Table 14

Mean (± SEM) FSH values (ng/ml) and temporal characteristics of the FSH peaks in each group for the 24 hr sampling period

FSH Parameter	Group 16L:18D KC	 16L:8D S	 6L:18D KC	 6L:18D S
Mean FSH	16.69 ± 2.50	17.92 ± 1.44	13.67 ± 0.61	15.23 ± 0.64
Maximum Peak Value	31.86 ± 5.15	27.40 ± 1.45	29.65 ± 1.90	36.63 ± 2.33
Time of Max. Value (hr)*	15.5 ± 1.19	14.0 ± 1.08	14.0 ± 0.58	22.0 ± 0.58 [a]
Peak Amplitude	17.12 ± 3.95	11.56 ± 1.05 [b]	18.01 ± 1.43	26.29 ± 1.97
Peak Duration (hr)	2.5 ± 0.87	4.3 ± 0.25	3.0 ± 0.0	2.33 ± 0.67
Time of Peak Onset (hr)*	14.75 ± 1.03	12.50 ± 0.87	12.67 ± 0.33	20.67 ± 0.33 [c]

[a] Significantly different from all other groups.

[b] Significantly different from 16L:8D KC and 6L:18D S groups.

[c] Significantly different from all other groups.* See text for description of hr values.

$p < 0.02$, vs animals with knife cuts in 16L:8D, $t\ (5) = -4.55$, $p < 0.01$, vs sham-operated animals in 16L:8D, $t\ (5) = -4.55$, $p < 0.01$. The mean time of peak onset for all three of these groups occurred significantly earlier during the 24 hr than for sham-operated animals in 6L:18D.

Likewise, analysis of the time of the maximum peak value also revealed a significant interaction, $F\ (1,10) = 22.10$, $p < 0.001$. There were also significant main effects for both photoperiod, $F\ (1,10) = 10.35$, $p < 0.01$, and surgical condition, $F\ (1,10) = 10.35$, $p < 0.01$. The time of the maximum peak value in sham-operated animals in 6L:18D occurred significantly later than for sham-operated animals in 16L:8D, $t\ (5) = -5.59$, $p < 0.01$, animals with knife cuts in 16L:8D, $t\ (5), = -4.55$, $p < 0.01$, or animals with knife cuts in 6L:18D, $t\ (4) = -3.95$, $p < 0.02$. No significant differences were found among any of the other groups ($p > 0.05$ for all comparisions).

Finally, a significant interaction was found for peak amplitude, $F(1,10) = 6.96$, $p < 0.02$. While there was no significant main effect of surgical condition for this variable, there was a significant main effect of photoperiod, $F\ (1,10) = 8.89$, $p < 0.01$. Follow-up analyses revealed that this interaction was due primarily to significantly higher peak values for animals with knife cuts in 16L:8D as compared with the sham-operated control group in 16L:8D, $t\ (6) = 4.54$, $p < 0.01$. The sham-operated animals in 6L:18D also had significantly higher FSH peak levels than sham-operated animals in 16L:8D, $t\ (5) = 3.88$, $p < 0.02$. There were no significant differences among any of the other groups ($p > 0.05$ for all comparisons). Thus, animals in 6L:18D tended to have higher peak amplitudes than animals in 16L:8D.

In order to further investigate the enhanced uterine weights seen in animals with knife cuts in both photoperiods, uterine weight measurements were correlated with mean FSH levels for all cannulated animals. The analysis for these animals did not reveal a significant correlation between uterine weight and mean FSH levels across the 24 hr sampling period ($p > 0.05$).

Discussion

As previously reported (see Experiment 1), knife cuts placed ventral to the PVN prevented the effects of exposure to short days on estrous cyclicity and uterine weight. In contrast to the findings in Experiment 1, however, the knife cuts in the present study not only prevented decreased uterine weight in short days, but also induced significantly higher uterine weights independently of photoperiod. The failure to find a significant correlation between uterine weight and mean FSH levels in the cannulated animals suggests that the enhanced uterine weight in animals with knife cuts is not due to the stimulatory effects of higher levels of FSH on E production. However, since FSH levels were determined for only a small subset of animals, this possibility cannot be entirely ruled out.

The findings for body weight gain in the present study also differ from the findings of Experiment 1 where neither knife cuts nor photoperiod had significant effects on body weight. However, in the present study, both groups of animals in short days gained more weight over the 11 week period than sham-operated animals in long days. Furthermore, animals with knife cuts in long days gained more weight than their respective sham-operated group. Lehman, et al. (1984) reported similar photoperiod-independent weight gains in male hamsters with lesions of the PVN housed in long days. In another report (Bartness, et al., 1985), lesions of the PVN blocked short-photoperiod induced weights gains in female hamsters fed a normal laboratory diet, but exaggerated weight gains and induced hyperphagia when animals were fed a high fat diet. In general, the weight gains induced by exposure to short days are not very robust, and are only found reliably for animals fed a high fat diet (Dr. M.H. Brown, personal communication). Body weight in past studies in our laboratory has generally followed a consistent, albeit nonsignificant, trend towards larger body weights in short days. In this context, the detection of a significant photoperiod effect in the present study is not surprising. and may merely reflect subtle

individual differences in responsiveness between the present animals and those from previous experiments.

Animals with knife cuts ventral to or through the ventral portions of the PVN in both photoperiods continued to show regular 4-day estrous cycles throughout the experiment. For animals in long days, the characteristics of the major FSH peak in animals with knife cuts sampled on the expected day of proestrous did not differ from those of sham-operated animals. Thus, it is likely that the FSH peak detected in the cannulated animals with knife cuts in both photoperiods reflects the proestrous FSH surge, and not a daily surge such as that seen for acyclic animals in either photoperiod (Bridges & Goldman, 1975; Jorgenson & Schwartz, 1984; Moline, et al., 1986). However, since the animals in this study were sampled during a single 24 hr period, the results do not exclude the possibility that these animals are also showing daily FSH surges. Future studies should investigate plasma FSH profiles in animals with knife cuts throughout the estrous cycle in order to ensure that FSH secretion in these animals is reflective of the normal 4-day pattern of secretion.

The temporal characteristics of the FSH peak did not differ between groups in long days. Both groups showed the onset of peak FSH secretion during the light portion of the light-dark cycle, approximately 4-5 hr before lights out. The time of the maximum peak was also similar, occurring 3-4 hr before lights out. In contrast, sham-operated animals in short days showed a delayed onset of the FSH peak to approximately 3 hr after lights out, and the maximum peak occurred 4-5 hr after lights out. The timing of the FSH peak for these animals is in agreement with previous reports for cyclic and acyclic females under these photoperiod conditions (Bridges & Goldman, 1975; Moline, et al., 1986). Interestingly, animals with knife cuts in short days showed the onset of the FSH peak and time of maximum peak that was significantly advanced as compared with sham-operated animals in the same photoperiod. In addition, the clock time at which this peak occurred was during the light portion of the light-dark cycle at a

time almost identical to that seen for both long day groups, i.e., approximately 4-5 hr before lights out.

The 4-day estrous cycle and cyclic gonadotropin secretion are under the control of a circadian mechanism (see Moore-Ede & Moline, 1985; Turek & Campbell, 1979, for reviews). When animals are exposed to stimulatory 24-hr light-dark cycles, the estrous cycle occurs over a 96 hr, or 4-day, period; however, under constant lighting conditions, the period of both the estrous cycle and pituitary gonadotropin release deviates from 96 hr and free-runs with a period that is four times that of the free-running circadian activity rhythm (Alleva, et al., 1971; Fitzgerald & Zucker, 1976). Previous work in male hamsters has shown that knife cuts placed ventral to the PVN block gonadal responses in short days, but do not disturb entrainment of the activity rhythm to the light-dark cycle (Nunez, et al., 1985). Since activity rhythms were not monitored in the present study, it is difficult to draw conclusions concerning the potential relationship between photoperiodic effects on the timing of FSH secretion and the activity rhythm in animals with knife cuts. However, if the knife cuts in the present study are permissive for the activity rhythm to maintain a normal phase relationship to the light-dark cycle in short days, as they do in males, the advanced FSH peak seen in these females may reflect a selective disruption of the circadian mechanism regulating the timing of FSH secretion.

In anestrous animals in short days, the daily LH and FSH surges become phase-shifted to a time closer to the onset of activity than is seen for the proestrus surge in cycling animals (see Moore-Ede & Moline, 1985, for a review). Unlike the delayed shifting of the FSH and LH surges during the transition to anestrous (i.e., several weeks), the onset of the activity rhythm phase-shifts by several hours almost immediately after exposure to short days. Future studies must evaluate other rhythms under circadian control, in addition to gonadotropin secretion, in order to elucidate the

potential role of a circadian mechanism in the altered timing of the FSH peak in animals with knife cuts.

Daily melatonin injections induce the appearance of daily gonadotropin surges in females housed in long days with a time course that closely resembles that seen after exposure to short days (Tamarkin, et al., 1976b). Melatonin may constitute the signal by which the hypothalamic-hypophyseal axis regulates the appropriate pattern of gonadotropin secretion under different photoperiods. In males, central continuous release implants of melatonin block the effects of short-day exposure on testicular size and circulating LH levels (Hastings, et al., 1988). These implants would provide central levels of melatonin high enough to obscure the phasic characteristics of the endogenous nocturnal melatonin peak that are believed to provide the signal for daylength information. These implants were only effective in mediobasal and anterior hypothalamic regions, and may have been acting directly upon GnRH neurons. It is possible that the prevention of photoperiodic effects on LH in that study, and FSH in the present study, were both due to disruption of the phasic characteristics of melatonin secretion.

Circulating levels of melatonin were not determined in the present study; however, if the knife cuts produce an effect similar to lesions of the SCN and the PVN, it is expected that the rhythm in pineal melatonin secretion was abolished. The circadian oscillator that generates the photic signal necessary for the regulation of rhythms in pineal melatonin secretion may have been intact in animals with knife cuts, but the cuts interrupted the pathway that is necessary for the transmission of this signal to the pineal gland.

An alternative explanation for the advance in the FSH peak may involve the effects of estrogen upon the timing of the FSH peak. Animals that have been exposed to short days for 2-3 weeks continue to show regular estrous cycles and normal profiles of both LH, FSH, and estrogen secretion, while progesterone secretion increases (Jorgenson &

Schwartz, 1985; Jorgenson & Schwartz, 1986). The surge of LH continues to occur during the light portion of the light-dark cycle (Jorgenson & Schwartz, 1986; Moline, et al., 1986). Furthermore, after exposure to short days for 8 weeks, the animals become acyclic, estrogen levels are greatly reduced, and the timing of the LH surge is delayed by approximately 4 hr as compared with values obtained after only three weeks of short-day exposure (Moline, et al.,1986).

Ovariectomized hamsters in short days also show delayed daily LH surges that can be advanced by treatment with estrogen. In the present study, the stimulated uteri of animals with knife cuts suggests that the animals had levels of estrogen higher than sham-operated controls. However, the timing of the surge did not differ between sham-operated animals in long days and animals with knife cuts in either photoperiod. Thus, there may be a threshold value of estrogen beyond which additional estrogen levels do not induce further advances. The temporal effects of estrogen on FSH secretion have not been investigated; however, it is possible that the advanced peak of FSH in animals with knife cuts in short days in the present study is due to a mechanism similar to that affecting the timing of the LH surge.

Estrogen has also been shown to affect the temporal characteristics of activity rhythms in ovariectomized hamsters (Morin, 1980; Morin, et al., 1977; Widmaier & Campbell, 1980). It is possible that the temporal effects of estrogen on LH, and possibly FSH, secretion are achieved via actions on the circadian system. Further studies of the role of steroids and the circadian system in modulating responses to photoperiod are necessary before the mechanism by which these knife cuts prevent gonadal and neuroendocrine responses to photoperiod is fully understood.

General Discussion

Many animals, particularly those within the temperate zones, display adaptive responses to seasonal changes in the environment that serve to promote the survival of both the species and the individual (Gwinner, 1981). Among these responses are changes in reproductive state, body coloration, metabolism, and body composition. While a number of external variables could convey information concerning changes in season (i.e., temperature, food availability, etc.), the most universal environmental cue used by mammals appears to be the light-dark cycle (see Gwinner, 1981, for a review).

The present series of studies sought to expand our understanding of the mechanisms by which reproductive responses to photoperiod occur within the context of the current model (see Tamarkin, et al., 1985, for a review) describing the functional anatomy of this system. However, rather than providing only corroborative evidence supporting this model, some of the present findings instead challenge the basic assumptions underlying it and encourage a re-evaluation of past interpretations based upon functional anatomical studies.

According to this model, the pineal gland, via the secretion of the endocrine product melatonin, is believed to provide critical information concerning daylength (see Elliott & Goldman, 1981; Goldman, et al., 1982, for reviews). However, a number of photoperiod-dependent seasonal changes do not appear to require the integrity of the pineal for their expression. Seasonal changes in body weight are partially independent of the pineal gland (Bartness & Wade, 1985), and the effects of short days on prolactin synthesis (Blask, et al., 1986) and sexual behavior (Experiment 2b) occur even in pinealectomized animals. Furthermore, photoperiodic effects on behavioral sensitivity to exogenous steroids are also not affected by administration of melatonin (Experiment 2c). Interestingly, the pineal-independent effects of photoperiod on prolactin secretion and sexual behavior are observed only in females. These findings encourage us to question not only the assumption that the pineal gland provides

the common signal for interpretation by various photoperiod-dependent systems, but also why these systems appear to be regulated differently in males and females.

In a sense, an explanation for these differences can not be achieved until we gain a better understanding of the nature of the photoperiodic signal itself. The duration of exposure to melatonin has been suggested as the critical parameter of the signal for interpreting daylength (Carter & Goldman, 1983). Nocturnal melatonin secretion is of a longer duration during the long nights of winter in long-day and short-day breeders alike. Despite this commonality, sheep (Bittman, 1984) and hamsters (Elliot & Goldman, 1981) are reproductively active at different times of the year. In contrast, two closely related species of long day breeders, Syrian and Turkish hamsters, respond differently to both pinealectomy and exogenous melatonin administration (see Goldman, et al., 1982, for a review). Finally, within a specific photoperiodic species, the Siberian hamster, testicular regression and pelage changes occur in response to different critical durations of melatonin secretion (Duncan, et al., 1985). These findings raise some interesting questions. If the daylength signal is sequestered within some aspect of the nocturnal melatonin pulse, how is it that long-day and short-day breeders use this signal to achieve reproductive competence at different times of the year? How can two species of long-day breeders demonstrate such markedly different responses to the same melatonin pulse? Furthermore, within a species, how can individual physiological systems, as well as males and females, respond differentially to the same signal?

The elusiveness of the answers to these questions may reside within the fact that the critical parameter of melatonin secretion that conveys daylength information has not yet been satisfactorily elucidated. The sex, species, and system differences described above suggest this shortcoming may result from attempts to find a "single" parameter common to all of these variables. It is possible, and indeed more likely, that each of these has evolved to attend to a specific, unique aspect of the signal that need not

compromise other responses. In addition, some of these systems may rely upon the same parameter, but have different thresholds for induction of the response, as is suggested by the findings for testicular regression and pelage changes in Siberian hamsters (Duncan, et al., , 1985).

While previous studies, as well as the present series of experiments, have focused upon the functional neuroanatomy of the system mediating photic regulation of pineal gland responses, less attention has been paid to the mechanism by which the systems "downstream" respond to these changes. The interpretations of functional results are of necessity limited by the number of endpoints measured in each study. It thus becomes possible for critical endpoints to be missed and misinterpretations of the results of functional studies can occur. For instance, a sex difference in the functional anatomy of the neural pathway mediating reproductive responses to photoperiod was suggested by the finding that knife cuts located dorsal to the PVN block gonadal responses to photoperiod in males (Eskes & Rusak, 1985; Inouye & Turek, 1986; Nunez, et al., 1985) but not females (Experiment 3). The effects of these knife cuts were originally interpreted to involve disruption of pineal melatonin secretion, despite the fact that pineal melatonin was never measured in these studies. When viewed in conjunction with the findings for the effects of these knife cuts on gonadotropin secretion, however, an alternative explanation arises; i.e., that the sex difference is really dependent upon differential responsiveness to the disruption of a neuroendocrine mechanism (Badura, et al., 1988) rather than a functional difference in PVN projections involved in regulation of the pineal gland. This example underscores the need for caution in interpreting functional data when the mechanism of interest, i.e., pineal function, is not evaluated directly.

Little is known about the sites or mechanisms of melatonin action. It is recognized, however, that melatonin production involves a circadian component (see Underwood & Goldman, 1987, for a review). The present results demonstrate that

interruption of the neural pathway responsible for transferring both photic and circadian information to the pineal abolishes gonadal responses to photoperiod (Experiments 1 and 4), while also preventing the effects of photoperiod on the timing of of the proestrous FSH surge (Experiment 4). However, although it is likely that the knife cuts in the present studies interfered with pineal responses to photoperiod, the actual mechanism by which gonadal maintainance is achieved remains unknown. Furthermore, the relative importance of disruption of circadian vs photic information cannot be fully investigated using the present techniques. Too frequently, the importance of more than one factor is not fully appreciated because too much emphasis is placed upon a single dependent variable; thus, we miss the forest for the trees. Future investigations of both the functional anatomy of this system, and the mechanisms by which photoperiodic responses are achieved, must include a broader sampling of photoperiod-dependent responses if we are to fully grasp the complexities of the interaction between environment and physiology necessary for the manifestation of seasonal reproductive cycles.

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